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Microplastic pollution in the aquatic environment

Murphy, Fionn

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1 **Microplastic Pollution in the Aquatic**
2 **Environment:**
3 **Sources, Destination & Effects**

4
5
6 Fionn Murphy

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8 Thesis submitted in partial fulfilment of the requirements
9 of the University of the West of Scotland
10 for the award of Doctor of Philosophy
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16 Date: 28 February 2017

Signature: Fionn Murphy

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Abstract

Microplastic are plastics that are < 5mm and is a contaminant of emerging concern in the aquatic environment. They are produced to be of a microscopic size or are created through the fragmentation of larger plastic material due to degradation. Microplastic has been found to be ubiquitous in the marine and freshwater environment with shoreline and deep sea sediment, oceans, rivers and lakes throughout the world observed to be polluted by microplastic. Wild populations of aquatic biota with various feeding behaviour have been observed to ingest microplastic. Exposure studies have also demonstrated the harmful effects of microplastic on a range of aquatic organisms. In this thesis, various aspects of microplastic pollution were investigated, from the sources of microplastic in the environment, to the destination of the microplastic once it enters the environment as well as the potential effects of microplastic on exposed organisms. The contribution a wastewater treatment works (WwTW) is making to microplastic pollution in the environment was estimated and the extraction efficiency of microplastic within the treatment process was determined. This study identified the key parts of microplastic removal in the treatment process. Aquaculture was also investigated as source of microplastic in the environment by comparing synthetic rope and netting used in the industry with microplastic extracted from cultured fish and wild shellfish. The ingestion of microplastic by a variety of fish species sampled from Scottish marine waters were investigated finding considerably higher ingestion rates in demersal flatfish sampled from coastal waters then species sampled further offshore in much deeper waters. The effects of microplastic were investigate by developing a novel bioassay to measure ecologically relevant endpoints such as feeding and reproduction as well as morphology in *Hydra attenuata* exposed to microplastic. *H. attenuata* feeding was found to decrease as microplastic concentration increased. This work shows that microplastic is ubiquitous throughout the aquatic environment and can potentially effect exposed organisms.

Authors declaration

This thesis has not been submitted for another comparable academic award elsewhere. Chapter 2, 4 and 5 of my thesis are presented as research papers (published or under review). I am the lead author on all three of these papers, all contributions from my co-authors are described at the beginning of each chapter.

Aims

The aims of my PhD thesis are to:

- Provide a detailed literature review of microplastic pollution in the aquatic environment
- Determine the microplastic removal rates at the different points of the wastewater treatment process and estimate the potential contribution a single wastewater treatment works is making to the microplastic load in the environment
- Investigate aquaculture facilities to determine the potential contribution they are making to microplastic pollution in the environment as well as investigating the ingestion of microplastic in cultured fish
- Investigate the ingestion of macroplastic and microplastic by demersal and pelagic fish species sampled from Scottish marine waters
- Develop a bioassay to test the effects of microplastic on a freshwater organisms feeding, morphology and reproduction

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Chapter 1

Introduction

1.1 Plastic

Plastic has become a vital part of modern life, in 1950 1.7 million tonnes of plastic was produced as of 2014 the worldwide production of plastic has been estimated to be 311 million tonnes (PlasticsEurope, 2015). Plastics represent a wide range of synthetic material that is malleable, persistent, lightweight and durable (Laist, 1987). These synthetic polymers are usually prepared by the polymerisation of monomers derived from petrochemicals (Shah et al., 2008) and often contain other chemical additives such as plasticisers (Wypych, 2004). There are two types of plastic thermoplastic and thermosetting plastic (Pascault et al., 2002). Thermoplastics soften when heated and then harden when cooled (Pascault et al., 2002) and account for 80% of all plastic produced. Thermoplastics are mainly used in packaging, as well as textiles and coatings (Al-Salem et al., 2009). While thermosetting plastics harden on heating and cannot be softened with subsequent heating (Pascault et al., 2002), thermosetting plastics are therefore much harder to recycle than thermoplastics (Pickering, 2006). Table 1.1 shows some of the most common types of plastic that are produced as well as some of their uses, due to the high production of these plastics they are also the most common types to end up in the environment.

Polyethylene terephthalate (PET) is used widely to manufacture plastic bottles as well as in the manufacturing of textiles and clothing. It is also widely used in the fishing and aquaculture industry as rope or netting. High and low -density polyethylene (HDPE) is by far the most produced plastic in the world due to the large demand, with various applications such as plastic bags and packaging. Polyvinyl chloride (PVC) is used to make pipes as well as electrical wire insulation. Polypropylene (PP) like PET is also widely used in the fishing and aquaculture industry as rope and netting. Polystyrene (PS) is often used in packaging and as insulation but can also be found in the marine environment as floating buoys. Other common plastics include polyamide or more commonly known as Nylon which is widely used in clothing as well as rope and netting and acrylic which is also used to make clothing as well as paint.

Table 1.1 Most common plastics produced and some of their uses, density of plastics taken from Quinn et al., (2017).

Resin Code	Description	Uses	Density g/cm ³
1 PET	Polyethylene terephthalate	Textile industry (clothing), Bottles, Rope, Netting, Food Containers	1.380
2 HDPE	High-density polyethylene	Bottles, Food Containers, Chemical Containers, Packaging	0.940 - 0.970
3 PVC	Polyvinyl chloride	Piping, Electrical Wire Insulation, Window Frames	1.100 - 1.450
4 LDPE	Low-density polyethylene	Laboratory Equipment, Plastic Bags, Packaging	0.915 - 0.925
5 PP	Polypropylene	Rope, Fibres, Food Containers	0.855 - 0.946
6 PS	Polystyrene	Insulation, Packaging,	0.960 -1.040
7 Other	Other Plastics such Polyamide & Acrylic	Clothing, Rope, Paint	Variable

The widespread use of plastic has resulted in plastics replacing more traditional materials such as glass, wood and metal in the manufacturing of goods and materials (Andrady & Neal, 2009) as in many cases they are lighter and more durable thus reducing costs (Shah et al., 2008). It is for these reasons amongst others that plastic has become such a ubiquitous material in every part of society. From food packaging, medical equipment and the construction industry plastic is important for most modern industries (PlasticsEurope, 2015). Indeed, a large proportion of plastic that is produced is used in packaging which is often single use (Eriksen et al., 2014). In Europe packaging accounts for 39.5% of all plastic demand (PlasticsEurope, 2015). The European Union produces 20% of the world's plastic behind China (26%) but ahead of NAFTA (19%) with demand increasing year on year (PlasticsEurope, 2015).

Although steps have been taken to increase the amount of plastic recovered and recycled, 38% of it still ends up in landfills in the EU which is still the first choice of disposal for many countries (PlasticsEurope, 2014). In the United States the amount of plastic ending up in landfills is considerable higher with 85.8% of all plastic ending up in landfills (Themelis et al., 2011), with much of the plastic waste persisting in the environment for many years (Singh & Sharma, 2008) and released into environment through accidental loss or by careless handling by individuals through littering (Wilber, 1987).

Plastic will invariably end up in marine and freshwater systems, indeed the presence of plastics in the environment has been widely reported (Derraik, 2002, Ivar do Sul & Costa, 2014) for many years. Plastic pollution has been observed to affect a range of species such as birds (Azzarello et al., 1987), turtles (Gramentz, 1988) and dolphins (Denuncio et al., 2011).

1.1.1 Sources

Due to many plastic being very light weight it allows them to be easily dispersed by wind across large areas of land (Laist, 1987). It is likely that much of this plastic is destined for rivers, lakes, or coastal areas, where the buoyant nature of many plastics allows it to be carried by ocean and river currents and dispersed even further (Laist, 1987). The source of plastic in the environment can vary depending on location. For example, 52% of litter found in a harbour in Canada was plastic with the majority originating from land based and recreational sources (Ross et al., 1991). While sampling of several beaches in New Zealand found virgin plastic used in plastic production to be occurring in large amounts (Gregory, 1977). Fishing vessels also contribute to plastic waste in the marine environment, through the abandonment or dumping of fishing gear such as ropes and netting as well as packaging material (Cawthorn, 1989, Matsuoka et al., 2005) (Figure 1.1). It has been reported for instance that up to 600,000 plastic containers were being deposited at sea every day by merchant ships (Horsman, 1982). As a considerable portion of plastic enters the environment, particularly the marine habitat (Thompson, 2006) a variety of species can encounter plastic in the wild with a number of negative effects.



Figure 1.1. Discarded fishing debris collected from a single beach in Scotland.

1.1.2 Impact

The aesthetics of a location can be compromised by the presence of plastic waste (Figure 1.2). The accumulation of debris may cause areas to become unsightly which could affect the number of visitors (Gregory, 1999). This can be particularly evident along beaches and shorelines where the lack of vegetation fails to mask the material that has accumulated (Gregory, 1977, Barnes et al., 2009). On the island of Kauai, Hawaii it was found that between 400 to 600 plastic fragments were deposited daily in one beach, over a period of ten days over 6000 pieces of plastic were collected (Cooper & Corcoran, 2010). The persistent washing up of this plastic debris on the shoreline would require constant collection to remove which may not be possible resulting in considerable plastic accumulation occurring.



Figure 1.2 Photograph of macroplastic & microplastic debris found washed up on a shoreline.

311 The interaction of macroplastics and marine biota has been widely studied, particularly
312 with seabirds and the more charismatic mega fauna such as turtles, cetaceans, and pinnipeds (Laist,
313 1997, Derraik, 2002). Marine plastic debris is recorded to affect at least 267 species worldwide,
314 this includes 86% of all sea turtle species, 44% of all seabird species and 43% of all marine
315 mammal species (Laist, 1997). Seabirds are particularly vulnerable to the effects of plastic
316 pollution, particularly in surface feeders as plastic debris can resemble prey items (Robards et al.
317 1995). Reports of seabirds ingesting plastic began being published over 50 years ago (Kenyon &
318 Kridler, 1969), when plastic production was a fraction of what it is currently. OSPAR has even
319 begun using the abundance of plastic in North Atlantic fulmars as an indicator of pollution
320 (Johnson, 2008). Fulmars appear to be particularly prone to the dangers of plastic pollution, 95%
321 of 1295 Fulmars sampled from the North Sea stomachs were found to contain plastic (Van
322 Franeker et al., 2011), with 58% of the birds sampled containing $\geq 0.1\text{g}$ of plastic. The ingestion
323 of plastic material may cause choking and a false sense of satiation (Ryan et al., 1988) which has
324 the potential to reduce the health of individuals.

325 The colour, shape and movement of a plastic item may cause it to be confused for a prey
326 item (Robards et al. 1995). Sea turtles have been observed ingesting floating plastic bags (Bugoni
327 et al., 2001). It is believed that they are confused for jellyfish which the turtles naturally prey on,
328 the plastic bag can become stuck in the mouth causing the animal to choke (Bjorndal et al., 1994)
329 or become entangled in the gastrointestinal tract potentially resulting in blockage or
330 gastrointestinal issues (Mrosovsky et al., 2009). Plastic ingestion by turtles can be quite high, a
331 study investigating plastic ingestion in loggerhead turtles taken from the western Mediterranean
332 found that out of 54 turtles sampled 75.9% had ingested plastic (Tomás et al., 2002).



Figure 1.3. Photograph of a green sea turtle entangled in derelict net. Source: NOAA
<https://marinedebris.noaa.gov/what-we-know-about-entanglement-and-ingestion>

Plastic also poses a threat to marine biota through entanglement in plastic packaging, synthetic rope, nets, lines and other discarded plastic material (Laist, 1997, Derraik, 2002, Gregory, 2009) (Figure 1.3). In the marine and freshwater environment plastic can remain floating for many years increasing the likelihood of a chance encounter with an animal (Laist, 1997). The nature of some plastic may make it difficult for an animal to spot it, if a plastic is clear in colour an animal may not be able to see it and could potentially swim into it and become entangled (Laist, 1997). Considerable focus has been paid to macroplastic pollution in the environment, however in the last 15 years attention has been turning to much smaller pieces of plastic collectively known as “Microplastic”.

1.2 Microplastic

Microplastics are plastics that are <5mm in size (Arthur et al., 2009) and have been overlooked in terms of their environmental impact until recent years (Thompson et al., 2004) but have quickly become a contaminant of emerging concern. Microplastics can be separated into two different types, primary microplastics and secondary microplastics.

1.2.1 Primary Microplastics

Primary microplastics are plastics that are manufactured to be of microscopic size these can be found in many personal care products as facial scrubbers (Microbeads) (Figure 1.4) (Zitko

& Hanlon, 1991, Fendall & Sewell, 2009 Napper et al., 2015). Due to the small size of these microbeads it's believed that when they enter the waste water stream their small size allows them to pass through the waste water treatment process (Fendall & Sewell). Napper et al., (2015) examined six different brands of personal care products containing microplastic and found a large variation in size ranges with one brand containing microplastics between 10µm to >2000 µm. Microplastics extracted from personal care products can occur in a variety of shapes such as uniform spheres, threads and irregularly shaped flakes (Fendall & Sewell, 2009 Napper et al., 2015) and usually consist of polyethylene (Fendall & Sewell, 2009 Napper et al., 2015). In recent years, significant public pressure has forced companies to remove microplastics from their products and governments to regulate and create legislation to ban the use of microplastics in personal care products (Doughty & Eriksen, 2013, Rochman et al., 2015, Girard et al., 2016). Microplastics are also used as an air blasting media to strip paint and clean engines (Gregory, 1996, Browne et al., 2007). Pre-production pellets (virgin plastic, plastic nurdles) used in the manufacturing of larger plastic material are normally between 2 to 5 mm in diameter (McDermid & McMullen, 2004) and have been recorded in the environment in a number of locations (Gregory, 1977, McDermid & McMullen, 2004) due to improper disposal and careless handling.

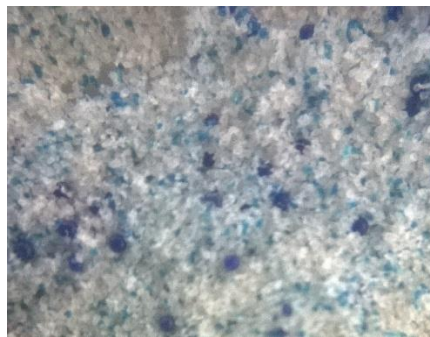


Figure 1.4 Polyethylene microbeads and flakes extracted from a cosmetic care product.

1.2.2 Secondary Microplastics

Secondary microplastics are formed from the breakdown of larger plastic debris (Singh & Sharma, 2008), which is slowly broken down via exposure to sunlight, wind, water and other environmental stressors (Ho et al., 1999, Singh & Sharma, 2008, Barnes et al., 2009, Webb et al., 2012) (Figure 1.5). Due to the durability of plastic it takes a considerable amount of time to breakdown in the environment, the length of time can vary depending on the plastic and the

environmental conditions but range from hundreds to thousands of years (Barnes et al., 2009). The degradation of plastic is caused by chemical, physical and biological reactions which cause bonds to break and subsequent chemical transformations (Shah et al., 2008, Singh & Sharma, 2008). Once plastic enters the environment it is exposed to ultra violet (UV) radiation from the sun which can cause the plastic to become brittle as the plastic undergoes photo-oxidation (Singh & Sharma, 2008). Photo-oxidative degradation is the process of decomposition of a material by the action of light (Zweifel, 1998). The majority of plastics are susceptible to degradation via exposure to UV and visible light which affects the soft segments of plastic, where photo irradiation generates ester, aldehyde, formate and propyl end groups (Nagai et al., 2005). The degradation of plastic by exposure to sunlight causes it to become brittle and in combination with the wind and wave action can cause the plastic to fragment into small pieces (O'Brine & Thompson). The level of degradation that occurs will vary depending on the type of plastic and the levels of light exposure (Singh & Sharma, 2008, O'Brine & Thompson, 2010). However, this susceptibility is severely reduced when plastics are submersed in seawater or landfills where they are buried and protected from light (Hamaide, 2014). Biodegradation is the transformation of a substance into different compounds by biochemical processes or microorganisms. Biodegradation may be enhanced by the other causes of degradation by increasing the surface area of the polymer allowing for greater microbial colonization or by reducing molecular weight (Shah et al., 2008). Although these processes do break plastic down over time, it is only into smaller fragments complete mineralisation of the plastic does not occur (Singh & Sharma, 2008). These smaller fragments, although difficult to see in most cases pose a threat to aquatic environments just on a much smaller size scale.



Figure 1.5. Photograph of partially degraded plastic (Unknown polymer) beach debris.

During the manufacturing process a variety of chemical additives are added to the plastic such as antidegradants, antioxidants, flame retardants, plasticizers amongst many others. These additives serve to imbue certain chemical properties to the plastic i.e. plasticizers to make it more flexible (Pritchard, 2012). These plastic additives could potentially leach out when released into the environment or when ingested by an organism and absorbed into the tissue. They also increase the durability of the plastic by reducing oxidation of the plastic. At present, it is not fully understood what the effects of these chemical additives are however experiments have shown that microplastic additives can leach out from microplastic once ingested (Browne et al., 2013).

1.2.3 Legislation Related to Microplastic Pollution

Marine litter has been a concern for many years, although it's only in recent years that focus has been placed on microplastic pollution there is existing legislation that relates to marine litter in general which would include microplastics. This section describes some of the important pieces of legislation that have been implemented to tackle marine litter as well as proposed legislation related to microplastics specifically.

The United Nations Convention on the Law of the Sea (UNCLOS) does not refer to marine litter but covers a range of issues related to the oceans such as environmental controls, however it does describe a general obligation on states to protect and maintain the marine environment (United Nations, 1982). The issue of litter discharged by ships is addressed by Annex V of MARPOL, developed by the International Marine Organization (IMO) essentially places a ban upon discarding waste at sea (MARPOL Annex V). The Honolulu Strategy formulated by the United Nations Environment Programme (UNEP) and the National Oceanic and Atmospheric Administration (NOAA) was developed with the aim to create a global framework on possible actions to tackle marine litter (NOAA & U.N.E.P., 2012). The development of a Global Initiative on Marine Litter by UNEP Regional Sea Programme and Global Programme of Action (GPA) has successfully organised and implemented various regional activities on marine litter throughout the world (UNEP, 2009). UNEP and the Intergovernmental Oceanographic Commission have developed guidelines for the surveying and monitoring of marine litter (Cheshire et al., 2009). The aim of these guidelines is to provide for long term scientific monitoring of marine litter. The Global Partnership of Marine Litter was also established by UNEP to act as a forum to coordinate activity amongst stakeholders to improve efficiency of resources.

The European Union (EU) has implemented a number of directives related to marine litter. The most important of which is the Marine Strategy Framework Directive (MSDF), this directive provides a framework with which member states can follow with the aim of achieving good environmental status in the marine environment by 2020 (EU, 2008). The Water Framework Directive (WFD) aims to achieve good water status for transitional waters and coastal waters (European Commission, 2010). The Port Reception Facility (PRF) Directive was implemented following on from the creation of MARPOL with the intent to reduce the contribution of waste produced by ships at sea (EU, 2000). The OSPAR Convention initiated in 1998, contains a number of Annexes related to the prevention and elimination of pollution from land based sources, by dumping or incineration and from offshore sources as well as assessment of the quality of the marine environment (OSPAR, 2000). OSPAR also has guidelines related to the monitoring of marine litter on beaches within the OSPAR Maritime Area.

The Microbead-Free Waters Act of 2015 implemented by the USA aims to specifically ban the manufacturing of cosmetics containing microbeads by 1st July 2017 and to ban cosmetics that contain intentionally-added plastic microbeads by 1st January 2018 (Microbead-Free Waters Act of 2015, (2015)). A government report by the United Kingdom has also made recommendations to ban the use of microbeads in cosmetic products, similar recommendations have also been made by the Canadian government.

1.3 Microplastics Sources & Routes into the Environment

Secondary microplastic enters the aquatic environment via a variety of sources but primarily from land based sources which contribute about 80% of plastic debris (Andrady, 2011). Careless handling of plastic litter by individuals or waste management systems can result in them being blown off shore or entering lakes and rivers (Wilber, 1987, Andrady, 2011), where exposure to the elements begins the process of degradation and fragmentation into microplastics (Singh & Sharma, 2008, O’Brine & Thompson). Plastic debris entering rivers will be carried out sea due to the unidirectional flow of these waterways (Moore et al., 2011). Legislation has been introduced in some countries in an attempt to minimise this consumer plastic waste, for example in Ireland the introduction of a tax on plastic bags reduced its use by 90% (Convery et al., 2007). However,

the substantial amounts of plastic produced will invariably lead to this material entering the environment.

Wastewater Treatment Works (WwTW) are also a source of microplastics in the environment. Microbeads used in facial scrubs, toothpaste and other personal care products will be washed down the drain and end up in a WwTW (Zitko & Hanlon, 1991, Fendall & Sewell, 2009 Napper et al., 2015) where they may potentially bypass treatment process due to their small size. Fibres released from washing synthetic clothing is also a concern as this can release thousands of fibres after just a single wash (Browne et al., 2011). The extent to which WwTW release microplastics may vary depending on the treatment level of the facility, treatment plants can use various treatment processes such as course & fine screening, sedimentation and biological treatment amongst other steps which may all have an on affect the level of microplastics released. Concentrations of microplastic measured previously in treated municipal effluents range from 0.0009 microplastics per litre (Carr et al., 2016) to 0.009 microplastics per litre (Magnusson & Noren, 2014) for secondary treatment and 0.000002 microplastics per litre (Carr et al., 2016) to 1 microplastic per litre (Browne et al., 2011) for tertiary level treatment facilities. This demonstrates the great variability in the amount of microplastic released from treatment facilities which may be due to the difficulty in sampling waste water treatment facilities (Ort et al., 2010). The amount of microplastic released from WwTW will also be influenced by the flow rate of incoming water, periods of heavy rain can overwhelm the treatment process forcing it to be released directly into the receiving water untreated. This has the potential to drastically increase the microplastic load of receiving waters during periods of heavy rainfall.

The commercial fishing sector is also a source of microplastics due to the high use of plastic material in this industry which utilises considerable amounts of synthetic line and rope (Ivar do Sul & Costa, 2014, Eriksen et al., 2014). The most commonly used fibres in the fishing industry consist of polyethylene, polyamide, polypropylene and polyester (Hameed & Boopendranath, 2000) which become frayed and damaged through use and exposure to the elements resulting in fragmentation. The loss or discarding of fishing line and nets can result in this material floating in the water column where it can continue capturing marine organisms in what is known as “ghost fishing” (Laist, 1996) or becoming washed up on beaches and shorelines degrading overtime. Commercial and recreational vessels also have a history of dumping waste material at sea

(Cawthorn, 1989, Matsuoka et al., 2005) although regulations are in place to prevent the discarding of plastic waste from vessels (MARPOL Annex V) this can be difficult to enforce at sea.

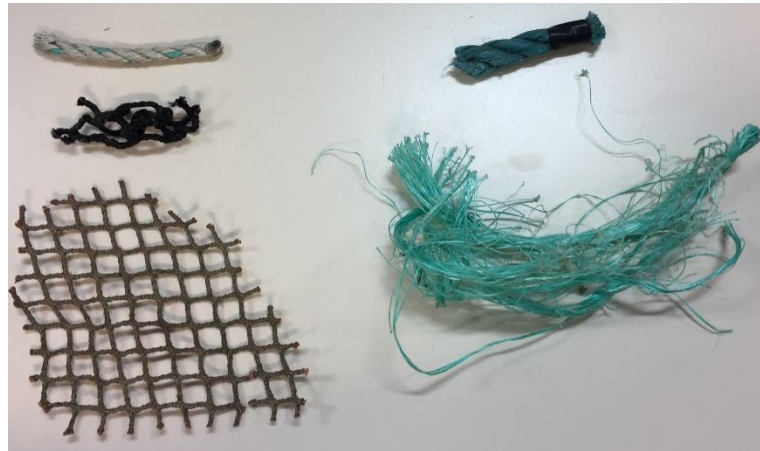


Figure 1.6 Photograph of a range of synthetic rope and netting used in the aquaculture industry.

Aquaculture may also be a source of microplastics in the environment due to it employing similar material as the fishing industry and the constant exposure to the elements likely causing degradation of this material (Figure 1.6). This is of concern due to the close proximity that cultured fish and shellfish will constantly be to this synthetic material. Fish may also nip or bite at the netting degrading it further. Aquaculture has been suggested as a source microplastic in the environment previously (Law & Thompson, 2014, Song et al., 2015) and styrofoam (expanded polystyrene) floating buoys used in aquaculture (Figure 1.7) have been observed to fragment and pollute shorelines in South Korea (Heo et al., 2013). Styrofoam pollution was also observed in areas of southern Chile, where 80% of marine debris consisted of polystyrene and was believed to be originating from aquaculture activity (Hinojosa & Thiel, 2009). Styrofoam fragments easily and can quickly produce large amounts of microplastics in a short period of time (Kusui & Noda, 2003).



Figure 1.7. Photograph of floating Styrofoam buoys in an oyster hanging culture farm (Heo et al., 2013).

Several studies have investigated the presence of microplastic in cultured shellfish (De Witte et al., 2014, Mathalon & Hill 2014, Van Cauwenberghe & Janssen, 2014, Li et al., 2016). A study in Nova Scotia, Canada investigated microplastic occurrence in wild and cultured *M. edulis* finding significantly higher amounts of microfibres in cultured *M. edulis* (average of 375 per 5 mussels) then in wild *M. edulis* (average of 170 per 5 mussels) (Mathalon & Hill, 2014). This could be the result of the use of synthetic line to act as a substrate for the growth of the mussels fragmenting and being ingested by the mussels attached to it. While wild mussels may be less likely to ingest microplastic as it has time to disperse before it reaches them.

The release of preproduction pellets into the environment by industrial plastic manufacturing is also significant contributor to microplastic pollution. The accumulation of pellets on beaches has been reported previously in Hawaii where preproduction pellets constituted 11% of the plastic waste found across 9 beaches (McDermid & McMullen, 2004). The outlet pipes of several plastic manufacturing plants were examined for plastic, finding polystyrene spheres between 1.0 mm to 13.3 mm were being released (Hays, & Cormons, 1974). While more recently Norén, (2007) reported high amounts of microplastic being released from a polyethylene production facility directly into a harbour in Sweden with concentration of $102\,000\text{ m}^{-3}$ reported between 0.5 to 2 mm in size. This single plant represents a major source of microplastics in the environment and demonstrates that a single source has the potential to heavily increase the microplastic load of nearby areas.

1.4 Destination

1.4.1 Sediment

The accumulation of microplastic on shorelines has been reported for many years (Gregory, 1977, Cawthorn, 1989), these early reports described the accumulation of preproduction plastic pellets on beaches in New Zealand measuring between 2 to 5 mm. While in more recent years' smaller types of microplastic < 2mm are being reported to be present in sediments throughout the world in marine, freshwater and estuarine sediments (Thompson et al., 2004, Ng & Obbard, 2006, Costa et al., 2010, Browne et al., 2010, Browne et al., 2011, Hidalgo-Ruz & Thiel, 2013, Imhof et al., 2013, Vianello et al., 2013, Leslie et al., 2013, Baztan et al., 2014, Castañeda et al., 2014, Klein et al., 2015, Nel & Froneman, 2015). Considerable sampling effort has been expended on beaches and shorelines due to the ease of access and have been the primary source of environmental abundance estimates. For example, Browne et al., (2011) examined sediment from 18 sites throughout the world representing 6 continents finding that every site examined contained microplastic. Microplastics identified primarily consisted of polyester, acrylic, polypropylene, polyethylene and polyamide fibres (Browne et al., 2011).

Several studies have investigated microplastic in Europe, Thompson et al., (2004) sampled sediment from around Plymouth, UK including sandy, estuarine and sub tidal areas finding that sub tidal areas had significantly higher amounts of microplastic present. Nine polymers were identified, including polyamide, acrylic, polyester and polyethylene consisting of fibres with similar types of polymers found within the water column (Thompson et al., 2004). The distribution of plastic debris was investigated along an estuary shoreline finding 952 plastic from 30 sediment samples of which 65% was microplastic, with plastic concentrations of between <1 to 8 items per 100 mL of sediment (Browne et al., 2010) (Figure 1.8.). Browne et al., (2010) reported greater quantities of plastic in downwind sites where less dense plastics were found in greater abundance. Lagoon sediment in Venice, Italy has also been examined (Vianello et al., 2013), finding concentrations of 672 to 2,175 items per kg⁻² dry weight (d.w.) with 82% of items consisting of polyethylene and polypropylene. A Belgium study investigated microplastic concentration on both the low and high tide line finding concentration of 9.2 items per kg⁻² d.w. (high tide) and 17.7 items per kg⁻² d.w. (low tide) with microplastic ranging in size between 38 µm to 1 mm and consisted of fibres and granules (Van Cauwenberghe et al., 2013a). Claessens et al., (2011) also

investigated microplastic in Belgium sediment sampled from beaches, harbours and sublittoral zones reporting average concentrations of 92.8 items per kg⁻² d.w. for beaches, 166.7 items per kg⁻² d.w. for harbours and 97.2 items per kg⁻² d.w. for sites sampled along the Belgium continental shelf the microplastic found were mainly fibres (59%) and granules (25%). Claessens et al., (2011) reported abundances considerably higher than Van Cauwenberghe et al., (2013a) and reflects the large spatial variation that can occur with microplastic within sediment. Sampling of sediment in Sweden revealed abundances of between 2 to 332 items per 100 mL and ranged in size between 0.5 to 1 mm in size (Norén, 2007).

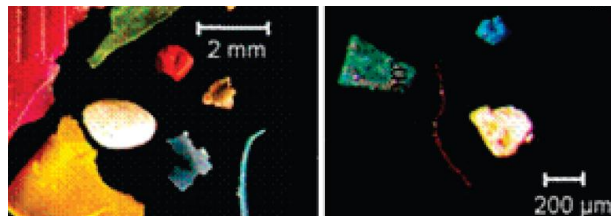


Figure 1.8. Plastic items collected from estuarine shorelines (Browne et al., 2010).

Microplastic abundances have also been reported in sediments from Asia. South Korean studies have reported concentrations of 913 items per m⁻² from the high strandline (Heo et al., 2013). While Lee et al., (2013) investigated differences in the abundance of microplastics (1 – 5 mm) between the dry season and rainy season on six beaches. Microplastic concentration in the sediment was higher during the rainy season (27,606 items per m⁻²) than in the dry season (8,205 items per m⁻²) with styrofoam the most common plastic found (Lee et al., 2013) and was thought to originate from buoys used in aquaculture similar to what was found by Heo et al., (2013). This demonstrates the seasonal variation that can occur due to differences in precipitation. Styrofoam particles were also found to be abundant in sediment from three sandy beaches on an isolated Korean island with total microplastic concentrations of between 56 to 285,673 items per m⁻² (Kim et al., 2015). While concentrations in beach sediment from Singapore have been reported as between 0 to 4 items per 250 g⁻¹ d.w. (Ng & Obbard, 2006) while a later study measured microplastic concentrations in sediment in several intertidal mangrove sites also in Singapore finding concentrations of between 3.0 per 250 g⁻¹ d.w to 15.7 per 250 g⁻¹ d.w (Nor & Obbard, 2014).

Sediment from the South African coastline has been investigated for microplastic (Nel & Froneman, 2015), with concentrations of beach sediment ranging between an average of 688.9 to 3,308 item per m⁻² and consisted of primarily blue and black fibres. Large scale sampling of 125 beaches on the Canary Islands off the coast of north western Africa reported concentrations by weight ranging between <1 to >100 g per L of sediment (Baztan et al., 2014).

Sediments from North America have also been examined for microplastics. Concentrations in Florida and Maine subtidal sediments have been reported as between 116 particles per L⁻¹ to 215 particles per L⁻¹ in Florida and 105 particles per L⁻¹ in Maine (Graham, & Thompson, 2009). Sampling of the top 5 cm of beach sediment from a beach in Hawaii, USA showed an average of 3.3% plastic by weight of sediment sampled with maximum of 30.2% observed (Carson et al., 2011). While a citizen science study carried out in Chile sampled beach sediment finding an average abundance of 27 plastic particles per m⁻² with 85% of items between 1mm to 4.75mm in size (Hidalgo-Ruz, & Thiel, 2013). This study also recorded microplastics concentrations in Easter Island with 805 particles per m⁻², which were considerably higher than beaches sampled in Chile (Hidalgo-Ruz, & Thiel, 2013). The high rates observed in the Easter Islands was likely due to surface currents of the South Pacific Sub Tropical Gyre transporting microplastic to the shoreline of the island (Hidalgo-Ruz, & Thiel, 2013, Eriksen et al., 2013b).

A number of studies have been carried out investigating microplastic in river sediments (Castañeda et al., 2014, Klein et al., 2015). Grabs samples were used to collect sediment from the St Lawrence River, microbeads were found in 8/10 of the sites sampled with an average of 13,759 ± 13,685 (SE) microbeads m⁻² across all sites found (Castañeda et al., 2014). The melting point of the microbeads was tested and suggested they were polyethylene and were similar to what was found by Eriksen et al., (2013a). While shoreline sediments taken from the River Rhine contained 228 to 3763 plastic particles per kg⁻¹ (Klein et al., 2015). The most abundant weight fraction were microplastics in the size range of 630 to 5000 µm, there was no significant correlation of the population density and the masses and numbers of microplastics at each sampling site (Klein et al., 2015). In Mongolia plastic density along the shoreline of a remote mountain lake varied from 37 to 5,324 g per km⁻¹ with microplastic accounting for 60% of this plastic by weight (Free et al., 2013). Fibres appear to be the most common type of microplastic found in sediments (Thompson et al., 2004, Browne et al., 2011 Claessens et al., 2011).

Deep sea sediments are also accumulating microplastics (Van Cauwenberghe et al., 2013b, Woodall et al., 2014). Sampling of 12 deep sea sediment sites from the Mediterranean Sea, southwest Indian Ocean and northeast Atlantic Ocean at depths of as much as 3500 m showed that microplastic were present at all sites investigated (Woodall et al., 2014). While Van Cauwenberghe et al., (2013b) found microplastic in 4 out of 6 sites sampled in the Atlantic Ocean and Mediterranean Sea at depths ranging from 1176 to 4844 m. These deep sea microplastics ranged considerably in size from between 2 to 3 mm (Woodall et al., 2014) and 75 to 161 μm (Van Cauwenberghe et al., 2013b). In the northwest Pacific Kuril-Kamchatka-Trench (Fischer et al., 2015), box corer samples were taken from depths ranging between 4,869 and 5,766 m. Concentrations within the sediment range from 60 items per m^{-2} to 2,000 pieces per m^{-2} with 75% of microplastics found consisting of fibres (Fischer et al., 2015). Limited sampling of the deep sea has been carried yet already there's evidence that deep sea habitats are accumulating microplastics, the potential impact of this microplastic pollution on deep sea organisms is not well researched.

This demonstrates the ubiquitous distribution of microplastic pollution in sediment. A variety of methods have been employed to extract microplastic from sediment making comparisons between studies difficult (Quinn et al., 2017). However, despite the lack homogeneity in sampling protocols it is clear that microplastics are ubiquitous on shorelines and beaches throughout the world (Ng & Obbard, 2006, Browne et al., 2011, Claessens et al., 2011).

1.4.2 Oceans, Rivers & Lakes

Microplastic concentrations in marine and freshwater bodies have been reported throughout the world. Sampling of surface waters using manta nets is one of the most commonly applied methods. These manta nets usually have a pour size of 333 μm but sampling methods can vary from study to study. Microplastic concentrations in marine waters have been recorded in the Pacific and Atlantic Oceans as well as in the Mediterranean Sea.

Sampling of the western English Channel revealed an average of 0.27 microplastic items m^{-3} (Cole et al., 2014), while sampling off the coast of Portugal showed there to be lower concentration of between 0.002 ± 0.001 to 0.036 ± 0.027 items m^{-3} depending on location (Frias et al., 2014). Sampling of equatorial Atlantic resulted in 1 item per 100 m^{-3} of seawater filtered to be present and consisted entirely of secondary microplastics (Ivar do Sul et al., 2013). Ivar do Sul et

al., (2014) later sampled seawater from around islands of the Western Atlantic finding microplastic particle concentrations ranging from 0.03 to 0.04 items per m^{-3} . Extensive sampling of the northeast Atlantic Ocean showed concentrations of 2.46 ± 2.43 plastic particles per m^{-3} with 89% of plastic items identified measuring $< 5\text{mm}$ (Lusher et al., 2014). Water samples taken from the coastal waters of South Africa were reported to have average concentrations of between 257.9 to 1,215 items per m^{-3} (Nel & Froneman, 2015).

Surface sampling of Arctic polar waters reported microplastic abundances of between 0 to 1.31 particles per m^{-3} with an average of 0.34 ± 0.31 particles per m^{-3} (Lusher et al., 2015b). Arctic sea ice may be a significant sink of microplastic and as this sea ice melts it could release substantial amounts of microplastic into the surrounding waters (Obbard et al., 2014). Obbard et al., (2014) analysed core samples from Arctic sea ice finding concentrations of 34 to 234 microplastic particles m^{-3} of ice. The ongoing decline in sea ice caused by climate change may result in significant amounts of microplastic trapped within the sea ice being released over the coming years (Parkinson & Comiso, 2013). Sub-surface samples were also collected at a depth of 6 m and a total of 150,000 L of seawater was filtered with an average of 2.68 particles per m^{-3} collected (Lusher et al., 2015b).

Collignon et al., (2012) sampled the Mediterranean Sea and discovered concentrations of 0 to 0.89 particles per m^{-2} with an average concentration of 0.116 particles per m^{-2} . A similar study found an average of 0.15 items m^{-3} in the central western Mediterranean Sea (de Lucia et al., 2014). C  zar et al., (2015) investigated plastic contamination in the Mediterranean and found 83% of plastic items recovered were below 5 mm. Microplastic concentrations of the North Pacific subtropical gyre have been reported as 0.116 particles m^{-3} (Goldstein et al., 2012) and between 0.021 to 0.448 particles m^{-2} (Goldstein et al., 2013). While sampling of the South Pacific subtropical gyre found an average of 25,000 particles km^{-2} (Eriksen et al., 2013b).

Estuarine surface waters of the Yangtze Estuary System, China were sampled with estuarine waters containing concentrations of between 500 particles per m^{-3} to 10,200 particles per m^{-3} (Zhao et al., 2014). Coastal waters were also sampled with concentrations considerably lower than the estuarine water with between 0.030 particles per m^{-3} to 0.455 particles per m^{-3} reported, indicating that rivers may transport significant amounts of microplastic into marine coastal waters (Zhao et al., 2014). A highly urbanised river in Chicago, USA was sampled upstream and

downstream of a WwTW (McCormick et al., 2014) using neuston nets (333 μm). Upstream concentrations of $1.94 (\pm 0.81)$ particles per m^{-3} and downstream concentrations of $17.93 (\pm 11.05)$ particles per m^{-3} were reported with the WwTW believed to be a point source of pollution causing the higher particle numbers downstream (McCormick et al., 2014). The concentrations reported by McCormick et al., (2014) are considerably lower than those found by Zhao et al., (2014). A similar study was carried out in Chesapeake Bay, USA in which four estuarine tributaries were sampled for microplastic (Yonkos et al., 2014). Concentrations ranged considerably from between $5,534 (\pm 5,134)$ particles per km^{-2} to $259,803 (\pm 60,150)$ particles per km^{-2} (Yonkos et al., 2014) with concentrations of microplastic being significantly positively correlated with population density.

A number of lakes have been examined (Eriksen et al., 2013a, Free et al., 2013, Imhof et al., 2013). In Mongolia, a study looked at the pelagic density of microplastic in a finding average density of $20,264$ particles per km^{-2} , however the specific polymers were not identified (Free et al., 2013). A similar study was conducted in the Great Lakes in North America (Eriksen et al., 2013a), this study sampled the Great Lakes using manta trawls (333 μm) and found an average concentration of $43,157$ particles per km^{-2} . The most populated lake was found to have the highest microplastic count. Proximity to urban areas has been linked to higher rates of microplastic concentrations in the environment (Sanchez et al., 2014). Lakes may be at greater risk of microplastic accumulation due to their closed nature while marine microplastic will be dispersed over a much greater area.

Studies measuring microplastic concentration in the water tend to examine the surface waters which will collect buoyant microplastics. However, when in the environment weathering of the microplastic and the formation of biofilms on the surface of microplastic has the potential to change its density which can result in the changes in the sinking behaviour of microplastics (Kowalski et al, 2016). Microplastic distribution within the water column will also be influenced by the hydrodynamic conditions of the environment they are present in. However, microplastic are abundant in marine and freshwater environments and occur in varying concentrations. Due to the ubiquitous nature of microplastic in marine and freshwater sediment and waters it is inevitable that aquatic organisms will interact with them in the wild.

1.4.3 Ingestion in Natural Populations

The ingestion of microplastics by marine and freshwater organisms has been reported to occur in a variety of species with varying feeding behaviours. Ingestion rates vary between species as well as locations with very high rates found in some areas and low rates in others. For example, 83% *Nephrops norvegicus* sampled from the west coast of Scotland were found to contain microplastic (Murray & Cowie, 2011) whereas 5.5% of pelagic and demersal fish examined from the North Sea and Baltic Sea had ingested plastic of some size with 74% of these items < 5 mm (Rummel, 2014).

Microplastic ingestion in 3 commercial demersal species was investigated from different Atlantic and Mediterranean Spanish marine regions, with 212 fish examined (72 dogfish, 12 hake, 128 red mullet) (Bellás et al., 2016). On average 1.56 ± 0.5 items per fish were found with 17.5% of fish ingesting microplastic with microplastic size ranging from 0.38 to 3.1 mm. Microplastic were mostly fibres (71%), spheres (24%), films (3.2%) and fragments (1.6%) and were mostly black (51%), red (13%) and grey (12.7%) in colour (Bellás et al., 2016). Another study in the Mediterranean Sea examined semi-pelagic *Boop boops* around the Balearic islands, Spain finding microplastic in 57.8% of all sampled *B. boops* with full and empty gastrointestinal tracts (Nadal et al., 2016), while full gastrointestinal tracts had microplastic in 67.7% samples. 731 items were observed in 195 full gastrointestinal tracts, ranging from 2.47 ± 0.23 to 4.89 ± 0.45 items per fish, with an average of 3.25 ± 0.25 and came in a range of colours (Nadal et al., 2016). Larger pelagic fish such as sword fish (*Xiphias gladius*), bluefin tuna (*Thunnus thynnus*) and albacore tuna (*Thunnus alalunga*) have also been proven to be ingesting microplastics in the Mediterranean Sea (Romeo et al., 2015) with ingestion ranging from 12.5% to 32.4% between the three species and consisted of white, yellowish, transparent and grey particles.

A number of studies have been carried out in the North Sea and Channel area (Foekema et al., 2013, Lusher et al., 2013, Rummel, 2014, Devriese et al., 2015). Brown shrimp (*Crangon crangon*) from the southern North and Channel area were found to contain 1.23 ± 0.99 microplastics per individual with 63% of samples containing microplastic (Devriese et al., 2015). While demersal and pelagic fish from the English Channel were found to contain microplastic in 36.5% samples with an average of 1.94 ± 0.10 microplastic items per fish (Lusher et al., 2013). However, ingestion rates varied considerably between species ranging between 23.5 to 51.9%

(Lusher et al., 2013). Foekema et al., (2013) found much lower rates of ingestion in a variety of fish species from the southern North Sea (5.4%) and the northern North Sea (1.2%) although similar rates of ingestion were observed in cod (*Gadus morhua*) sampled from the English Channel (33%). Two species of invertebrate (*Mytilus edulis* and *Arenicola marina*) sampled from six locations along the French, Belgian and Dutch coastline were shown to be ingesting microplastic (Van Cauwenberghe et al., 2015). Ingestion rates were higher in *A. marina* with 1.2 ± 2.8 particles per gram of tissue than *M. edulis* with 0.2 ± 0.3 particles per gram of tissue, however fibres were excluded from the microplastic counts due to the lack of contamination controls put in place (Van Cauwenberghe et al., 2015).

While in the northeast Pacific Ocean analyse of zooplankton has shown that they too are ingesting microplastics (Desforbes et al., 2015). Average amounts of microplastic ingested ranged from 0.026 ± 0.005 particles per copepod (*Neocalanus cristatus*) to 0.058 ± 0.01 particles per euphausiid (*Euphausia pacifica*) with microplastic ingestion higher closer to shore (Desforbes et al., 2015). Mesopelagic fish sampled from the North Pacific subtropical gyre were also found to be ingesting microplastic with 9.2% of fish ($n = 141$) containing microplastic with an average length of 2.2 ± 1.9 mm in their stomachs (Davison & Asch, 2011). Goldstein & Goodwin, (2013) also examined microplastic ingestion in the North Pacific subtropical gyre finding that 33.5% of the barnacle species they examine had ingested microplastic with a median size of 1.41 mm. These microplastics consisted mostly of polyethylene (58.4%), although 35% of the microplastics were unable to be identified due to melting during analysis (Goldstein & Goodwin, 2013).

Studies have also been carried out in estuarine waters particularly in Brazil (Possatto et al., 2011, Dantas et al., 2012, Ramos et al., 2012). Three species of catfish ($n = 60$ for each species) were found to have ingestion rates ranging between 18% to 33% (Possatto et al., 2011). A study carried out in the Goiana Estuary, Brazil found that 7.2% of two drum species ($n = 569$) were found to contain plastic (Dantas et al., 2012). Three species of Gerridae ($n = 425$) representing juveniles, sub-adults and adults were examined for plastic, 13.4% of individuals contained plastic (Ramos et al., 2012), for all three of these Brazilian estuarine studies the plastic identified consisted of blue nylon fragments originating from fishing activity in the area.

There has been some research on the uptake in freshwater organisms in natural populations (Faure et al., 2012, Sanchez et al., 2014, Biginagwa et al., 2016). A preliminary study carried out

in France looked at the occurrence of microplastics in 186 wild gudgeons sampled from 11 streams (Sanchez et al., 2014). Of the fish sampled 12% contained microplastic and was only detected in urban rivers. While 20% of Nile perch and Nile tilapia purchased in a harbour market in Lake Victoria were found to contain microplastic however the sample number was quite small ($n = 20$) for both species (Biginagwa et al., 2016). Other areas such as Lake Geneva have been investigated for the uptake in wild populations (Faure et al., 2012). Despite the presence of both macroplastic and microplastic in the beach surrounding the lake and in the surface water no polymers of any size were found in the 51 fish examined (Faure et al., 2012). Much higher rates of ingestion have been observed in two sunfish species (*Lepomis macrochirus* & *Lepomis megalotis*) in a river in the Brazos River Basin, Texas (Phillips, & Bonner, 2015), where 45% of the 436 fish analysed contained microplastic 96% of which were threads.

Cultured and wild mussels have been investigated previously for the presence of microplastics (De Witte et al., 2014, Mathalon & Hill 2014, Van Cauwenberghe & Janssen, 2014, Li et al., 2016). An average of 0.36 ± 0.07 particles g^{-1} in *M. edulis* and 0.47 ± 0.16 particles g^{-1} in *Crassostrea gigas* was observed in North Sea coastal locations (Van Cauwenberghe & Janssen, 2014). However, this study utilised acid digestion (69% nitric acid) to breakdown organic matter and filter out any microplastics present which could potentially degrade microplastics that are present in the samples (Claessens et al., 2013, Van Cauwenberghe & Janssen, 2014). A study in Nova Scotia, Canada investigated microplastic occurrence in wild and cultured *M. edulis* finding significantly higher amounts of microfibrils in cultured *M. edulis* (average of 375 per 5 mussels) then in wild *M. edulis* (average of 170 per 5 mussels) (Mathalon & Hill 2014), however visual identification was used which has the potential to overestimate the amount of microplastic present (Hidalgo-Ruz et al., 2012, Rocha-Santos & Duarte 2015). Wild *M. edulis* sampled from the west coast of Scotland that were investigated for microplastic ingestion using enzymatic digestion were found to contain 1.05 ± 0.66 to 4.44 ± 3.03 microplastic particles g^{-1} wet weight mussel tissue (Courtene-Jones et al., 2017), which is higher than what was found in cultured *M. edulis* (Van Cauwenberghe & Janssen, 2014), these differences in the amount of microplastic present could be attributed to the different treatment techniques used to extract the microplastics from the sample tissue.

Whales have also been investigated for the ingestion of microplastic (Fossi et al., 2012, Besseling et al., 2015, Lusher et al., 2015a, Fossi et al., 2016). Three True's beaked whales (*Mesoplodon mirus*) found stranded on the coast of Ireland had their gastrointestinal tracts analysed for microplastic (Lusher et al., 2015a), finding that one of the specimens examined contained 88 microplastic particles. Microplastics have also been recovered by a humpback whale (*Megaptera novaeangliae*) stranded in the Netherlands (Besseling et al., 2015). Studying the ingestion of microplastic by large marine mammals is challenging due to the reliance on stranding events to determine ingestion.

The ingestions of microplastic by such a variety of different species with varying feeding strategies and habitats demonstrates the ubiquitous nature of microplastic in the environment. Microplastics ingested are primarily fibres and have been thought to originate from fishing activity. Although there is considerable evidence of wild aquatic biota ingesting microplastic the impact of this ingestion is not well studied it is therefore difficult to know the potential impacts of this widespread ingestion.

1.5 Exposure Studies: Ingestion & Effects

The effect of microplastic on aquatic biota is not fully understood, it is believed it could have similar effects to ingestion of macroplastic by larger organisms such as seabirds (Robards., 1995) but on a smaller scale. Potential effects include choking, internal damage, blockage of the gastrointestinal tract, false sense of satiation and the transfer of other harmful environmental contaminants and the leaching of contaminants from the plastic once ingested. Although a number of studies on the effects of microplastic have been carried out there is tendency in these studies to expose test organisms to unrealistically high concentrations of microplastic that would not be found in the environment. Although these exposures may not always be environmentally relevant they do demonstrate the great variety of organisms with the potential to ingest this emerging contaminant.

A range of zooplankton species collected from the Baltic Sea exposed to microplastic (10 µm polystyrene microspheres) were reported to ingest microplastic (Setälä et al., 2014). Another zooplankton exposure study by Cole et al., (2013) investigated the ingestion in several zooplankton

species sampled from the English Channel and tested the effects of microplastic on feeding rates on the copepod *Centropages typicus*. Thirteen of the fifteen zooplankton species exposed were found to be capable of ingesting polystyrene beads (7.3 – 30.6 μm), while *C. typicus* exhibited significantly reduced feeding on algae when exposed to high concentrations of polystyrene beads (Cole et al., 2013). This study demonstrates the potential risk that some of the smallest organisms face in relation to microplastic pollution. It also raises important questions about the knock on effects this could potentially have on aquatic biota further up the food chain.

A number of studies have been carried out on the effects on *Mytilus edulis* (blue mussel). Browne et al., (2008) exposed mussels to 0.5 g L^{-1} polystyrene (2 μm & 4 – 16 μm) microparticles and found that after 12 hours these microparticles were accumulating in the gut cavity and digestive tubules and after 3 days they were also found within the haemolymph. This study demonstrated the potential for microplastics to translocate from the gut after ingestion to the circulatory system. Another study exposing mussels to polystyrene microparticles (10, 30 & 90 μm) measured the effects on energy metabolism and found no significant difference between the control and mussels exposed to 110 particles mL^{-1} (Van Cauwenberghe et al., 2015). Although there was a 25% increase in energy consumption in the digestive gland of exposed mussels (Van Cauwenberghe et al., 2015). Von Moos et al., (2012) showed that mussels are also capable of ingesting high density polyethylene (HDPE) particles (0 – 80 μm) and that this resulted in a strong inflammatory response in digestive gland tissue after 6 hrs of exposure and significant disruption in lysosomal membrane integrity after 96 hrs. The effects on feeding behaviour were investigated on mussels exposed to 30 nm polystyrene microspheres combined with algae (Wegner et al., 2012). This study observed reduced algae feeding in mussels exposed to polystyrene (Wegner et al., 2012). Filter feeding organisms may be at a greater risk of microplastic pollution due to their indiscriminate feeding behaviour and the large amounts of water they can filter. This could result in the accumulation of microplastic overtime time even if environmental microplastic is present in low concentrations.

The polychaete worm *Arenicola marina* (Lugworm) has also been the used in determining the effects of microplastics. Ingestion was reported by Thompson et al., (2004) in lugworms while lugworms exposed to 1.5 g microplastic L^{-1} and spiked sediment (110 particles g^{-1}) and seawater (110 particles mL^{-1}) with 10, 30 & 90 μm polystyrene microspheres were observed to have no

significant effect on exposed lugworm energy levels over the 14 day exposure (Van Cauwenberghe et al., 2015). However, lugworms exposed to 5% UPVC spiked sediment over a four week period were observed to have significantly reduced feeding and energy reserves by up to 50% compared to the control (Wright et al., 2013).

Several crustacean species have been exposed to microplastics to determine effects. Welden & Cowie, (2016) investigated the impact of large fibre aggregations on the health and mortality of *Nephrops norvegicus* over an 8 month exposure trial. Three groups of 12 were used in the 8 month trial (fed 1.5g squid mantle seeded with 5 polypropylene fibres, the fed control group of 1.5g squid mantle only and the starved control group). Polypropylene fibres measured between 3 to 5 mm in length and 0.2 mm in diameter. Gut content analysis showed aggregations of 0.41 to 3.49 mg (average 1.5 mg). The starved group showed the highest mortality (58.3%), followed by the plastic fed (41.6%), and then the fed individuals (33.2%). The group fed plastic significantly reduced growth (-0.0189%) compared to the control (0.0795%). The plastic fed groups were observed to have lower feeding rates compared to the control. There was reduced nutrient uptake based on the haemolymph. Relative high water levels in the haemolymph indicated reduced lipid content in the plastic and starved groups.

Trophic level transfer has been observed in shore crabs (*Carcinus maenas*) that have been fed mussels exposed to 0.5 μm fluorescent polystyrene microspheres (Farrell & Nelson, 2013), these microspheres were capable of being transported into the haemolymph and tissue of the crab. This demonstrates an indirect route of ingestion of microplastics in organisms which is a concern for organism particularly those that prey on filter feeders as they are more likely to accumulate microplastics. Watts et al., (2014) observed ingestion and retention of polystyrene microspheres (8 – 10 μm) within the foregut of shore crabs as well as the uptake of microspheres in the gills of the crab. While an exposure investigating the effects of microplastic inspiration on shore crab fitness showed polystyrene microspheres were accumulating on the gills of the crabs (Watts et al., 2016). Oxygen consumption was significantly lowered in the highest concentration used (10^7 microsphere L^{-1}) one hour after but no difference after 16 and 24 hours (Watts et al., 2016). Although there were no significant adverse effects to the crabs it does demonstrate the potential effects microplastic can have even if no ingestion occurs.

A number of studies have looked at the potential uptake and effects of microplastics on freshwater organisms in the laboratory, these include invertebrate and vertebrate species (Rosenkranz et al., 2009, Imhof et al., 2013). Imhof et al., (2013) exposed a range of freshwater invertebrate species to microplastic and found 5 freshwater species capable of ingesting microplastic. *Daphnia magna* exposed to 20 nm and 1000 nm fluorescent polystyrene microsphere were found to uptake the spheres at concentrations of 2 µm per litre (Rosenkranz et al., 2009). When placed in clean water after 4 hrs of exposure 90% of the 1000 nm microspheres were cleared from the *Daphnia* after 4hrs and only 40% of the 20 nm in the same time period. This suggests that size plays an important part in the retention rate of microplastics (Rosenkranz et al., 2009). The potential impact of microplastics on fish is not fully understood, but negative effects have been observed in fish larva exposed to environmentally relevant concentrations of polystyrene microspheres (Lönnstedt & Eklöv, 2016). European perch (*Perca fluviatilis*) larva exposed to environmentally relevant concentrations of microplastic were found to exhibit lower hatching rates, lower activity rates and lower survival rates compared to the control (Lönnstedt & Eklöv, 2016). The use of environmentally relevant data is of great importance in assessing the potential threat that wild organisms face. More effects study need to be carried out using environmentally relevant data in order to identify species or habitats at greatest risk to the threat of microplastic.

1.6 Microplastic Co-contaminants

The physical effects of microplastics on aquatic biota is not the only concern, there is also the threat of microplastics acting as a vector for other harmful contaminants in the environment. These contaminants can concentrate onto the surface of microplastic potentially increasing its toxicity (Heskett et al., 2012). These contaminants can concentrate onto the surface of the microplastic and following ingestion can expose aquatic biota to that contaminant resulting in harmful effects. The process by which contaminants leach from or concentrate on to microplastics will depend on the specific contaminant and polymer of the microplastic. Some polymers may be more susceptible to the sorption of contaminants than others. For example, Non-polar plastics such as polyethylene and polypropylene will have a greater affinity for hydrophobic compounds such as persistent organic pollutants (POPs) (Crawford & Quinn, 2016). As plastic breaks down due to exposure to the elements it's surface area increases resulting in increased surface area for the

sorption of contaminants. For Example, polypropylene exposed to ultraviolet radiation for up to 7 weeks exhibited cracking on the surface (Figure 1.9) (Yakimets et al., 2004). Weathered plastic has also been shown to release leachate under simulated conditions (Bejgarn et al., 2015).

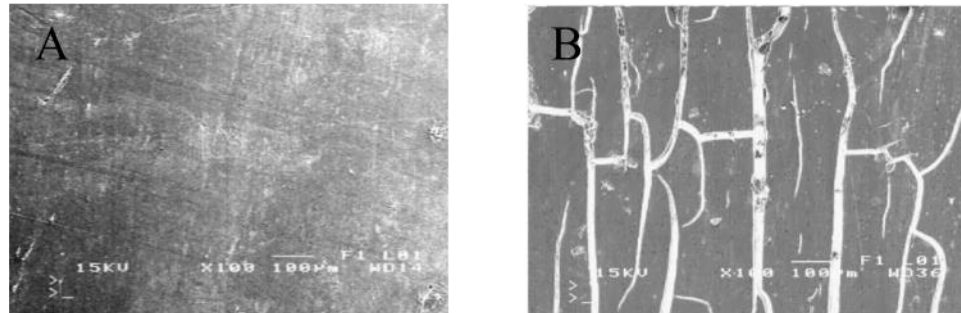


Figure 1.9 Scanning electron microscope images of polypropylene exposed to ultraviolet light for (A) 0.5 weeks (B) 6 – 7 weeks (Yakimets et al., 2004).

Studies have been carried out to test the absorption of various contaminants such as POPs, polychlorinated biphenyls (PCBs) and persistent bioaccumulative and toxic substances (PBTs) on to microplastic (Besseling et al., 2013, Rochman et al., 2013). Heavy metals often originating from anti-fouling paint also have the ability to sorb onto the surface of microplastic. Two heavy metals, copper and zinc from paint have been observed to leach out from paint and sorb on to virgin polystyrene and aged PVC (Brennecke et al., 2016). The threat of microplastic co-contaminants is an area that is under researched and of vital importance as microplastic ingestion in wild populations of aquatic biota is widespread.

The absorption of POPs and PCB onto the surface of microplastics has been shown to occur in a number of studies however much less research has been carried out on subsequent desorption. The ability of microplastic co-contaminants to desorb once ingested is of great importance due to the ever increasing number of organisms proven to be ingesting microplastics in the wild. Bakir et al., (2014) demonstrated the desorption of microplastic co-contaminants in simulated gut conditions for both warm and cold blooded conditions, this study showed that desorption could be up to 30 times higher in seawater. Japanese medaka exposed to both virgin and marine low density polyethylene (LDPE) over a two month period displayed signs of liver stress (Rochman et al., 2013). After two months of dietary exposure there was a greater concentration of PBTs in fish exposed to the marine-plastic treatment. Sever glycogen depletion was observed in 74% of marine

plastic exposed fish, 46% of virgin plastic fish and 0.5% of control fish. Fatty vacuolation was observed in 47% of marine plastic fish, 29% virgin plastic fish and 21% of control fish. Single cell necrosis was also observed in 11% of marine plastic fish and 0% of the virgin plastic and the control fish. Common goby exposed to pyrene (20 and 200 μm) for 96hrs in the presence and absence of microplastics (0, 18.4 and 184 $\mu\text{g L}^{-1}$) (Oliveira et al., 2013). Microplastic combined with pyrene exposure decreased the energy available through the aerobic pathway of energy production. Besseling et al., (2012) exposed *A. marina* to polystyrene with sorbed PCB contaminated sediment for 28 days with a low polystyrene dose of 0.074% increasing bioaccumulation of PCBs by a factor of 1.1 to 3.6.

These studies demonstrate the risks that microplastic co-contaminants may have on aquatic organisms. Microplastics have the potential to act as sink of environmental contaminants resulting in them concentrating on to the surface of the microplastic (Bakir et al., 2012). These sorbed contaminants may subsequently be released from the microplastic once ingested resulting in toxic effects to the exposed organisms.

Chapter 2

Wastewater Treatment Works (WwTW) as a source of microplastics in the aquatic environment.

Abstract

Municipal effluent discharged from wastewater treatment works (WwTW) is suspected to be a significant contributor of microplastic (MP) to the environment as many personal care products contain plastic microbeads. A secondary WwTW (population equivalent 650,000) was sampled for microplastics at different stages of the treatment process to ascertain at what stage in the treatment process the MP are being removed. The influent contained on average 15.70 (± 5.23) MP. L⁻¹. This was reduced to 0.25 (± 0.04) MP. L⁻¹ in the final effluent, a decrease of 98.41%. Despite this large reduction we calculate that this WwTW is releasing 65 million microplastics into the receiving water every day. A significant proportion of the microplastic accumulated in and was removed during the grease removal stage (19.67 (± 4.51) MP/2.5g), it was only in the grease that the much publicised microbeads were found. This study shows that despite the efficient removal rates of MP achieved by this modern treatment plant when dealing with such a large volume of effluent even a modest amount of microplastics being released per litre of effluent could result in significant amounts of microplastics entering the environment. This is the first study to describe in detail the fate of microplastics during the wastewater treatment process.

This chapter is a reformatted copy of my publication: **Murphy, F., Ewins, C., Carbonnier, F. and Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environmental science & technology, 50(11), pp.5800-5808.** I was lead author on this paper and carried out all sample analysis and identification. Ewins, C. assisted with sample identification, Carbonnier, F. provided information and data on the sampling site, Quinn, B. assisted with the experimental design and sample collection. All co-authors provided comments and edits to create the final manuscript.

2.1. Introduction

Plastic pollution in the aquatic environment is well studied and has been given considerable attention for a number of decades (Azzarello & Van Vleet, 1987, Pruter, 1987, Derraik, 2002). Due to the light weight nature of plastic it is easily dispersed by wind and ocean currents across vast distances (Laist, 1997). In recent years, the issue of small plastic particles known as microplastics has been gathering increasing attention (Andrady, 2011). Microplastic are plastics that are <5mm in size (Arthur et al., 2009) and can be separated into two different types, primary microplastics and secondary microplastics. Primary microplastics are plastics that are manufactured to be of microscopic size these can be found in many cosmetic products where they are used as facial scrubbers or as an air blasting media (Gregory, 2009). Secondary microplastics are formed from the breakdown of larger plastics debris (Thompson et al., 2004), via exposure to sunlight, wind, water, and other environmental stressors (Singh & Sharma, 2008).

Microplastics are ubiquitously found in aquatic water bodies (Collignon et al., 2012, Eriksen et al., 2013a) and sediments (Claessens et al., 2011, Van Cauwenberghe et al., 2013b) and have been ingested by various aquatic organisms (Murray & Cowie, 2011, Cole et al., 2013, Lusher et al., 2013). Trophic level transfer of microplastics has also been shown to occur (Farrell & Nelson, 2013). Due to their small size microplastics may be more bio available to lower trophic organisms (Farrell & Nelson, 2013), who tend to display limited selectivity and will often ingest anything of appropriate size (Moore et al., 2001). While organisms of higher trophic levels may ingest microplastics indirectly through trophic level transfer via their prey or by mistaking microplastics for a prey item (Wright et al., 2013).

However, despite this ubiquitous nature, the sources of microplastics in the environment are not fully understood. Wastewater Treatment Works (WwTW) could potentially be a major source of microplastics in the aquatic environment (Browne et al., 2007). Microbeads used in facial scrubs, toothpaste and other personal care products are transported in the raw effluent to WwTW (Fendall, & Sewell, 2009, Chang, 2015), where due to their small size they may bypass the waste treatment process. In recent years increased public pressure has led companies and governments to regulate and ban the use of microbeads (Rochman et al., 2015). Synthetic clothing such as polyester and nylon is also a concern as these fabrics can shed thousands of fibres into the wastewater (Browne et al., 2011).

The growing issue of microplastics released from WwTW was recently reported by the Norwegian Environmental Agency (Sundt et al., 2014). This report highlighted the knowledge gap regarding the analysis of microplastics discharged from WwTW, particularly entering river systems and the need for analysis of the fate and removal of microplastics during the treatment process. The report also highlighted the need for more detailed analysis of microplastic particles in order to classify them based on the polymer, size and type. Some research has been undertaken on microplastics in WwTW final effluent (Browne et al., 2011, Magnusson & Norén, 2014, Carr et al., 2016), but little work has been undertaken to determine their removal efficiencies and at what stage in the process microplastics are extracted and determining the composition of the polymers entering and exiting these treatment facilities.

There has been some research carried out on microplastics in WwTW (Browne et al., 2011, Magnusson & Norén, 2014, Carr et al., 2016). These studies have mainly focused on the final effluent, with little work done on determining removal efficiencies and where in the process microplastics are extracted. A Swedish study investigated the ability of a WwTW to retain microplastics and found that 99% of the microplastic was removed from the final effluent (Magnusson & Norén, 2014). However, this was a relatively small WwTW serving 12,000 people and limited identification was conducted on the specific polymers found. While Carr et al., (2016) looked at microplastics from secondary as well as tertiary WwTW by filtering large volumes of effluent as well as attempting to analyse different stages of the treatment process. Identification of MP was primarily visual using MP extracted from personal care products as a visual reference with limited FT-IR conducted. Browne et al., (2011) also examined effluent from a tertiary WwTW finding 1 MP. L⁻¹, however only small 750 ml samples were filtered. Concentrations of MP measured previously in treated municipal effluents range from 0.0009 MP. L⁻¹ (Carr et al., 2016) to 0.009 MP. L⁻¹ (Magnusson & Norén, 2014) for secondary treatment and 0.000002 MP. L⁻¹ (Carr et al., 2016) to 1 MP. L⁻¹ (Browne et al., 2011) for tertiary. However, comparison between these concentrations is made difficult due to the variable sampling techniques and identification methods employed.

In this study, we investigate the effectiveness of the WwTW process in the removal of microplastic from municipal effluent at different stages during the treatment process of a large secondary WwTW with a population equivalent of 650,000. We identify where in the treatment

process microplastics are being removed, identify the physical and chemical composition of the microplastics found at each treatment stage. We provide the first systematic overview of the fate of MP in municipal treatment plant, identifying and quantifying where MPs are removed at various stage of the treatment process. There are three separate aspects to this study, the examination of (i) Liquid fraction (ii) Solid fraction: comprising of grit, grease and sludge cake (SC) (iii) 24 hr SC duplicate: SC sampled at two different time points on two consecutive days

2.2. Materials & Methods

2.2.1 Sampling

A large secondary WwTW located on the River Clyde, Glasgow was sampled for microplastics at different stages of the treatment process. This site has the population equivalent of approximately 650,000 and produces on average 260,954 m³ of treated wastewater every day that is discharged into Glasgow's major waterway, the river Clyde. Samples were taken after coarse screening in order to avoid larger debris clogging or damaging the equipment used to filter the samples. Four stages of the treatment process were sampled (Figure 2.1A): Influent after 19 mm coarse screening (S1), grit & grease effluent (S2), primary effluent (S3), and the final effluent (S4) before it is released to the river Clyde (see Supporting Information (SI) Figure S2.1 for detailed description of the treatment process).

Steel buckets (10L) attached to steel wire were lowered into the turbulent effluent stream (<50cm) for sample collection by an on-site technician. The sample was then passed through steel sieves (65µm) to collect any debris present. Due to the large amount of debris it was only possible to filter 30L (3 x 10L pooled sample) from sites 1 – 3 and 50L (5 x 10L pooled sample) from site 4 before the sieves became clogged. This debris was then washed into clean glass bottles using distilled H₂O and all equipment was cleaned using on-site hoses between samples. All samples were taken in duplicate. The bottles were then sealed and brought to the laboratory, where the samples were vacuum filtered through Whatman No. 1 qualitative circles, 90mm filter paper, with a pore size of 11µm.

Samples of grit and grease were taken from the grit and grease removal stage (Figure 2.1), and SC from the sludge centrifuge treatment for comparison of microplastics present in the solid

effluent fraction from the WwTW. On a separate day, SC samples were also collected at the centrifuge treatment stage (Figure 2.1) at 09:30AM and again at 14:30PM on two consecutive days in order to determine any variation in the amount of microplastic present.

2.2.2 Contamination Mitigation

A number of steps were taken to reduce the incidence of microplastic contamination. During these steps clean white cotton lab coats were worn at all times, only natural fabric and no clothing made from synthetic fibres was worn underneath the lab coats.

2.2.2.1 Cleaning

All equipment used was cleaned three times with distilled H₂O. All petri dishes, filter papers and forceps were examined underneath a dissection microscope before use to ensure no contamination was present. All work surfaces were wiped down with 70% ethanol three times prior to work commencing.

2.2.2.2 Taping

The tape lifting techniques use in forensic science laboratories to check laboratory benches for fibre and particle contamination was used in this study (Wheeler & Stanclffe., 1998). The tape consists of a plastic film with one side covered with a layer of glue and is placed so that the glue makes contact with the area being examined. The tape is then lifted and any trace particles present should adhere to the tape, which is then placed on a clean sheet of acetate. To take a taping a piece of tape measuring 5cm x 5cm was randomly placed three times on the work surface after it was cleaned. After all lab work was completed another taping was taken and was also placed on a sheet of acetate. The tapings were then examined under a microscope for identification. Tapings were carried out before and after all procedures. On average 3.3 fibres per taping taken were collected ranging from 0-14 fibres per taping.

2.2.2.3 Atmospheric Microplastic (MP)

Clean filters in petri dishes were left out for the duration of the filtration in order to collect any atmospheric MP that may be present. Before the liquid fraction filtering began 750ml of distilled H₂O was vacuum filtered, and the filter examined for contamination. Clean filters in petri dishes were also left out for the duration of lab work and were then checked for any contamination.

2.2.3 Analysis

Samples were vacuum filtered and all bottles containing the liquid fraction were rinsed three times with distilled H₂O and filtered after each rinse. The filter was then observed under a dissection microscope. Initially, all debris present was considered to be microplastic until proven otherwise by FT-IR, as relying solely on visual identification is open to bias (Hidalgo-Ruz et al., 2012, Rocha-Santos & Duarte, 2015). Following the FT-IR identification large amounts of material could be discounted (e.g. plant material) and the microplastics were removed and characterised based on their colour, length and type (fibre, bead, flake...etc.). Due to the large amount of debris on the filters from sites 1-3, it was neither practical nor viable to identify all material present. It was therefore necessary to take sub samples from these sites. It should also be noted that due to the complexity of the samples items of similar colour to the background filter paper may have been overlooked.

To subsection the samples from sites 1-3 the filters were divided into 24 pie sections, and numbered 1-24. Using a random number generator, four sections were selected for each filter. These sections were then excised using scissors and thoroughly analysed for microplastics. An average of the four sections was used to get an estimate of the amount of microplastics present for the whole filter. Using the equation below an estimated amount of microplastics released at each site was made.

$$MP\ Released = \left(\frac{MP\ Present}{Litres\ Filtered} \right) \times Average\ Vol.\ of\ Effluent\ Released\ (Litres/Day)$$

The solid fraction samples of grit, grease, SC and SC 24 hr duplicate were mixed thoroughly for 1 min before taking a 2.5g sub sample in triplicate. Initially a larger quantity was examined but due to practical constraints and the time and effort needed to analyse these samples, a homogenous representative sub-sample of 2.5g was chosen. This sample was left to dry at a low heat <50°C for 2 hours, examined and analysed using a dissection microscope as above and the amount of microplastics present per 2.5 gram was determined.

2.2.4 Identification: Fourier Transform Infrared (FT-IR) spectrometry

A dissection microscope was used to separate out and collect material for identification by FT-IR analysis with any microplastic identified being photographed. A Perkin Elmer Spectrum One FT-IR Microscope was used in the reflection mode using gold coated glass microscope slides. Infrared radiation from $600 - 4000\text{cm}^{-1}$ was used, with 16 scans taken to produce the spectra, a variable aperture size was used and the spectral resolution was 4 cm^{-1} . FT-IR allows the identification of chemical bonds present in the samples and gives a characteristic signal in the “fingerprint” region. Samples are identified with the aid of reference spectra library (SI Figure S2.2). However, these reference spectra represent very clean and ideal samples, not typically found in the environment. It was therefore deemed necessary to create a more representative library of non-typical reference plastics taken from various sources such as beach debris, recycled waste and microbeads from face washes amongst others. This allowed a comparison to much more environmentally relevant samples to be made. As well as using reference spectra to make identifications the presence of characteristic functional group signals at the correct wavenumber values were checked to confirm the likely chemical structure of materials being examined.

2.2.5 Statistical Analysis

Statistical analysis was conducted using R statistical computing software. Differences in the number of microplastics and their sizes between sites were determined using one-way ANOVA's. Log10 transformation was used to transform data relating to the number of microplastics present in order to meet the assumptions of normality and equal variance. Size data did not need to be transformed as it already met the assumptions needed to carry out an ANOVA. A Pearson correlation analysis was conducted on the stage of treatment and the amount of MP. L⁻¹ present.

2.3. Results

In total 430 plastic items were identified across all the samples examined, the majority of which came from the liquid fraction (n=303), followed by the solid fraction (n=79) and 24hr SC duplicate (n=48) samples. Of the 430 items identified as plastic, 8 were >5mm.

2.3.1 Liquid Fraction

There was a significant difference in the amount of microplastic (MP) found between the four sampling sites ($p = 0.0002$) (Figure 2.1B). The influent sampled at S1 contained on average $15.70 (\pm 5.20)$ MP. L⁻¹ which was reduced by 98.4% in the final treated effluent sampled at S4 to $0.25 (\pm 0.04)$ MP. L⁻¹ (Table 2.1). Despite the highly efficient removal rate, using three years of flow rate data from the WwTW an estimated 65,238,500 MP could be released from the WwTW every day in the final effluent or 23 billion microplastics annually from this WwTW alone (Table 2.1). S2 (grit & grease removal) showed the biggest reduction in the amount of microplastic at 44.59%, this was further reduced by the primary settlement tanks by an additional 33.75%. Aeration & clarification reduced the amount by 20.07% before the effluent was released into the receiving water.

There was a significant negative correlation between the treatment stage and the number of MP. L⁻¹ ($p = 0.014$). The most common polymers found in S1 were alkyds (28.7%), polystyrene-acrylic (19.1%), polyester (10.8%), polyurethane (8.9%) and acrylic (8.3%) (Table 2.2). The most common polymer found in the final effluent (S4) was polyester (28%), polyamide (20%), polypropylene (12%), acrylic (12%), alkyd (8%), polyethylene (4%), polystyrene (4%) and PET (4%) (Table 2.2).

The liquid fraction contained mainly flakes (67.3%), fibres (18.5%), film (9.9%), beads (3.0%) and foam (1.3%) (Figure 2.2A). The sampling process may have resulted in the number of flake items being overrepresented as these flakes were very brittle and fragmented easily during identification. There was no significant difference between the sizes of plastics found at each site ($p = 0.913$). The MP found were predominantly red (26.7%), blue (25.4%) and green (19.1%) but other colours were also present (Figure 2.2B).

2.3.2 Solid Fraction & 24hr SC Duplicate

There was a significant difference between the number of MP/2.5g in the three different solid fractions investigated ($p = 0.002$) (Figure 2.1C). The grease sample contained an average of $19.67 (\pm 4.51)$ MP/2.5g sample, which was significantly higher than both the grit sample ($p = 0.009$) and the SC sample ($p = 0.002$). From the 24hr SC duplicate study, there was no significant difference in the number of MP/2.5g SC found between the Day 1 and Day 2 or 09:30 and 14:30

($p = 0.383$), or between the time of day the samples were taken (Figure 2.1D). Polyester, acrylic, polypropylene, alkyd, and polystyrene were the most commonly found polymers in the 24hr SC duplicate study (Table 2.2).

2.3.3 Size Comparison

There was a significant difference in the size (mm) of MP found between the liquid fraction, solid fraction, and 24hr SC duplicate study ($p = 0.002$) (Figure 2.1E). MP taken from the liquid fraction were on average 0.598mm (± 0.089) in size and were significantly smaller than both the solid fraction 1.342mm (± 0.519) and the 24hr SC duplicate study 1.618mm (± 0.394) ($p = 0.002$). There was no significant difference between the solid fraction and the 24hr SC duplicate study ($p = 0.4$). There was no significant difference in the sizes of the microplastics between the times sampled in the 24hr SC duplicate study ($p = 0.782$).

2.3.4 Contamination

Throughout the course of the study 25 items were found to have accumulated on the filters that were left out to test for atmospheric microplastic contamination. Following FT-IR examination just one item was identified as a microplastic (polyester), with the rest identified as blue/black cellulose/cotton fibres with a very distinctive ribbon like morphology when examined under light microscope (SI Figure S2.3). Similar fibres were also found on the tapings taken and following observation under light microscope these were also identified as cellulose/cotton.

2.4. Discussion

2.4.1 Liquid Fraction

Preliminary and primary treatment effectively removed 78.34% of the microplastics from the liquid fraction. Preliminary treatment involves the removal of large items such as rags and sticks as well as the removal of floatables, grit and grease that may damage or interfere with the equipment used in the treatment process (Tchobanoglous & Burton et al., 1991). Primary treatment involves the removal of a portion of the suspended solids and organic matter, achieved through the use of chemical additives (flocculation agents) and sedimentation (Waite, 1999). The secondary treatment stage managed to remove a further 20.1%. Secondary treatment involves the removal of

biodegradable organic matter as well as suspended solids during the aeration and clarification treatment (Figure 2.1A) (Tchobanoglous & Burton et al., 1991).

Chemicals such as ferric sulphate are used in the treatment process in order to cause suspended particulate matter to aggregate together forming a “floc” (Waite, 1999). It is likely that the amount of ferric sulphate or other flocculating agents will have an effect on the particulate matter present in the wastewater. For the WwTW in the current study the amount added is flow dependent but on average a total of 7-9 g/m³ are used, with primary treatment receiving 2 g/m³ and secondary treatment getting 5-7 g/m³ during aeration (Figure 2.1A). Polyacrylamide is also used as a flocculation agent (Figure 2.1A), this is a white water soluble powder. Samples of this were taken and examined in order to exclude it from the final results. Bacterial jelly-like balls are also formed at the aeration stage, likely aiding in the accumulation of particulate debris.

A study conducted by the Swedish Environmental Research Institute found high removal rates of 99% in a smaller WwTW serving a population equivalent load of 12,000 (Magnusson & Norén, 2014). Although microplastic polymer composition was not fully described as the identification of items was mainly visual, several items were identified using FT-IR and included polyester, polyethylene, and polypropylene. This study estimated that 2,000 microplastics were released in the effluent on one particular day, equivalent to 0.16 MP/Person/Day or 0.009 MP. L⁻¹. This microplastic concentration is considerably lower than the 100 MP/Person/Day or 0.25 MP. L⁻¹ of final effluent found in the current study. While a concentration of 1 MP. L⁻¹ was found in the effluent from two tertiary treatment plants in New South Wales, Australia (Browne et al., 2011). This comparatively high concentration was surprising considering the additional treatment process involved in tertiary treatment. However direct comparison is difficult as the specific treatment processes, the volumes of effluent treated and the population equivalent was not described in this study. A recently published study carried out in Southern California also examined effluent from several tertiary and a secondary WwTW (Carr et al., 2016), 423,000 L of effluent were filtered at the secondary WwTW and 373 MP were counted or 0.0009 MP. L⁻¹. However this study conducted limited FT-IR, identification was mainly visual and relied upon comparison of MP derived from personal care products. This may have resulted in MP being underestimated, while the number of MP found in the tertiary effluent was even lower with the highest count being 0.000002 MP. L⁻¹ at one site. This study also attempted to investigate the

transport of microplastics at each stage of the tertiary treatment process. But owing to practical issues with clogging of filtration equipment (sieves) and the use of density separation, only 5 L of the raw influent was examined with no MP identified making determining reduction rates difficult. Therefore the current study is the first to provide detailed data on the removal rates of MP at various stages of municipal effluent treatment as well as the detailed characterisation of MP found. The concentrations reported in these four studies are all considerably lower than what was reported in a Dutch survey of microplastics in the environment (Leslie et al., 2013). The concentrations reported ranged from 9 MP. L⁻¹ to 91 MP. L⁻¹, averaging 52 MP. L⁻¹ however identification of microplastic was visual so the concentrations are likely to have been overestimated.

The three most common polymers found in the final effluent in the Australian study were polyester (30.4%), polyamide (21%), and acrylic (13%) the same as in the current study. Browne et al., (2011) reported the presence of only fibres in municipal effluent which is in contrast to the various different types of microplastics found in the current study. This is most probably due to the large difference in the sizes of the two facilities but may also be influenced by the difference in influent composition from the surrounding catchment, the degree of urbanisation and the time of day that sampling was conducted as well as the specific treatment process used at that facility.

2.4.2 Solid Fraction

Analysis of the solid fraction (grit, grease and SC) samples showed high amounts of microplastic accumulating in these three stages. This was most evident in the grease stage, which showed a significantly higher amount of microplastic present. It was only from the grease samples that the much publicised microbeads from face washes were found (Fendall & Sewell, 2009) (Figure 2.3). It has been suggested that due to their small size, microbeads are capable of passing through the coarse and fine screens (designed to remove large debris to prevent damage to the equipment) and through the wastewater treatment process without removal. The microbeads found in the majority of face washes consist of polyethylene (www.BeattheMicrobead.org), which is positively buoyant in water and is likely to sit on the surface of the wastewater where it can be easily skimmed off the surface layer during grease removal. In this study no microbeads were found in the final effluent, indicating that microbeads from face washes may not be a major issue for the receiving environment if appropriate treatment processes are implemented. However, owing to the small sample size, this study may not be entirely representative. Microbeads from

face wash products have been previously found in the Great Lakes in North America (Eriksen et al., 2013a), but due to the size of the area studied it is difficult to determine their source.

Certain aspects of the grit and grease removal stage implemented in this WwTW are site specific and may not be normal practice in other locations. Typically, at this stage of the treatment process skimmers are placed on the surface of the effluent to skim off any floating grease, while the grit portion settles at the bottom. The WwTW sampled in the current study uses aeration to cause frothing of the grease, making it more likely to be collected and removed from the effluent. On average 12-15m³ of grease is removed from the effluent each day. This is then incorporated along with the grit to the sludge (Figure 2.1A) where it is then sent for incineration in a waste to energy scheme.

Synthetic material has previously been found in sludge samples (Habib et al., 1998, Zubris & Richards, 2005) as well as effluent samples (Browne et al., 2011, Dubaish & Liebezeit, G., 2013). A study examining the presence of synthetic fibres in WwTW sludge found 4 fibres per gram of sludge sampled (Zubris & Richards, 2005). However, this study only examined synthetic fibres as an indicator of soil pollution and may have resulted in other non-fibrous polymers being overlooked.

2.4.3 24hr SC duplicate

The 24hr duplicate study showed no difference in the amount of microplastic present in the sludge over this time period investigated. However, this study may have used too short a time between sampling periods to provide a solid conclusion and it would be more appropriate to look at longer time frames such as a monthly comparison and more frequent sampling in future studies.

2.4.4 Destination

Microplastic size was considerably smaller in the liquid fraction than in the solid fraction and 24hr SC duplicate study samples. This may be due to the smaller items remaining suspended within the liquid fraction, while the larger items are more likely to settle at the bottom of settlement tanks or be captured in the grit & grease stage. It could also be due to only the smallest items being capable of passing through the treatment process. The final destination of these microplastics released in the treated effluent remains unknown but evidence suggests they may be accumulating in the river banks of the Clyde or carried out into the estuary and eventually the Clyde Sea. A

preliminary study conducted by Habib et al., (1998) examined sediments collected from a bay downstream of a sewage treatment plant. It was found that the sediment contained numerous synthetic fibres and as distance increased from the sewage treatment plant the size and number of fibres decreased. The difference in concentrations of microplastics up and downstream from a WwTW has also been examined (McCormick et al., 2014) finding a higher downstream concentration of MP (17.93 m³) compared to the upstream concentration (1.91 m³), with primarily fibres and fragments being found. The river Clyde receives the effluent from a number of WwTW which could all be contributing to the microplastic load. Microplastics have been previously identified in the Clyde Sea, with 83% of *Nephrops* examined found to contain microplastics (Murray & Cowie, 2011), although it was thought that these were primarily sourced from discarded fishing line and rope.

2.4.5 Contamination

An important aspect of this study was the implementation of various contamination controls to ensure the validity of the findings. Contamination has been put forward as a topic of concern in microplastic research (Browne et al., 2011, Hidalgo-Ruz et al., 2012). Similar methods to reduce and determine the incidence of contamination such as avoiding wearing synthetic clothing, thorough cleaning, the use of filters to collect atmospheric microplastics, as well as forensic taping techniques were also developed for microplastic sediment analysis (Woodall et al., 2015). Through the use of these contamination controls it was determined that the incidence of microplastic contamination from clothing or atmospheric particulate matter is very small provided appropriate controls are put in place. The methods used in the current study are simple, cheap and require little technical training to carry out but do require care to be taken to prevent contamination. Implementation of these contamination controls in future microplastic research should be included to provide additional validity to the results obtained.

2.4.6 Limitations of this study

Sampling in wastewater treatment systems presents a number of challenges as reviewed by Ort et al., (2010). This review examined the study of pharmaceuticals and personal care products but should also be applicable to sampling for microplastics. The review highlights practical limitations in sampling such as environmental and the daily variability of flow rates as well as variability in pollutant concentration. In future studies the time of day, year and weather patterns

should all be considered when sampling. Due to the great variation of flow rates it may be more appropriate to take frequent samples throughout the day rather than taking a snapshot as was done in this study.

This current study did not take into account storm water runoff, where untreated effluent is released directly into the river when the volume of incoming water exceeds the treatable volume. According to flow rate data taken from the WwTW, when averaged out over the year 39,000 m³ of effluent with limited treatment (settlement in storm tanks) is released every day or potentially an additionally 620 million microplastics/day using the figure of 15.70 MP. L⁻¹ taken from S1. However, this normally occurs in large volumes across short periods of time during spells of bad weather, for example on one particular day over 700,000m³ was recorded to have been released as storm water. This untreated wastewater may potentially heavily increase the amount of microplastic entering the receiving environment. However, it's important to take into account the dilution factor that would occur, although the volume of wastewater increases it is unlikely to increase the amount of microplastics present. Although large storm tanks used to hold excess untreated wastewater, allow some settlement to occur reducing the amount of particulate matter and the amount of denser microplastic present before being released/treated, the issue of storm water overflow has yet to be investigated in relation to microplastic contamination.

2.5. Conclusion

The results of this study show that WwTW can be effective in the removal of microplastic from the municipal effluent. However even a small amount of microplastic being released per L⁻¹ can result in significant amounts of microplastics entering the environment due to the large volumes being treated. These treatment processes are standard wastewater treatment practices and are implemented worldwide. The study goes some way to determine what the most important steps in the treatment process are in the removal of microplastics i.e. grit & grease removal and primary settlement and to address the knowledge gaps highlighted by the Norwegian Environmental Report (Sundt et al., 2015). Treatment facilities where these particular processes are less efficient may be making a greater contribution to microplastic pollution in the environment. This will provide important information in the reduction of microplastic pollution in guiding waste management processes. It is also important that future research on microplastics in WwTW takes a site specific

approach by detailing any unique practices carried out by the WwTW studied. More research is needed to determine the difference between the ability of primary, secondary and tertiary WwTW to remove microplastics as well as the potential temporal differences in the release of microplastics from this source into the environment.

Acknowledgements

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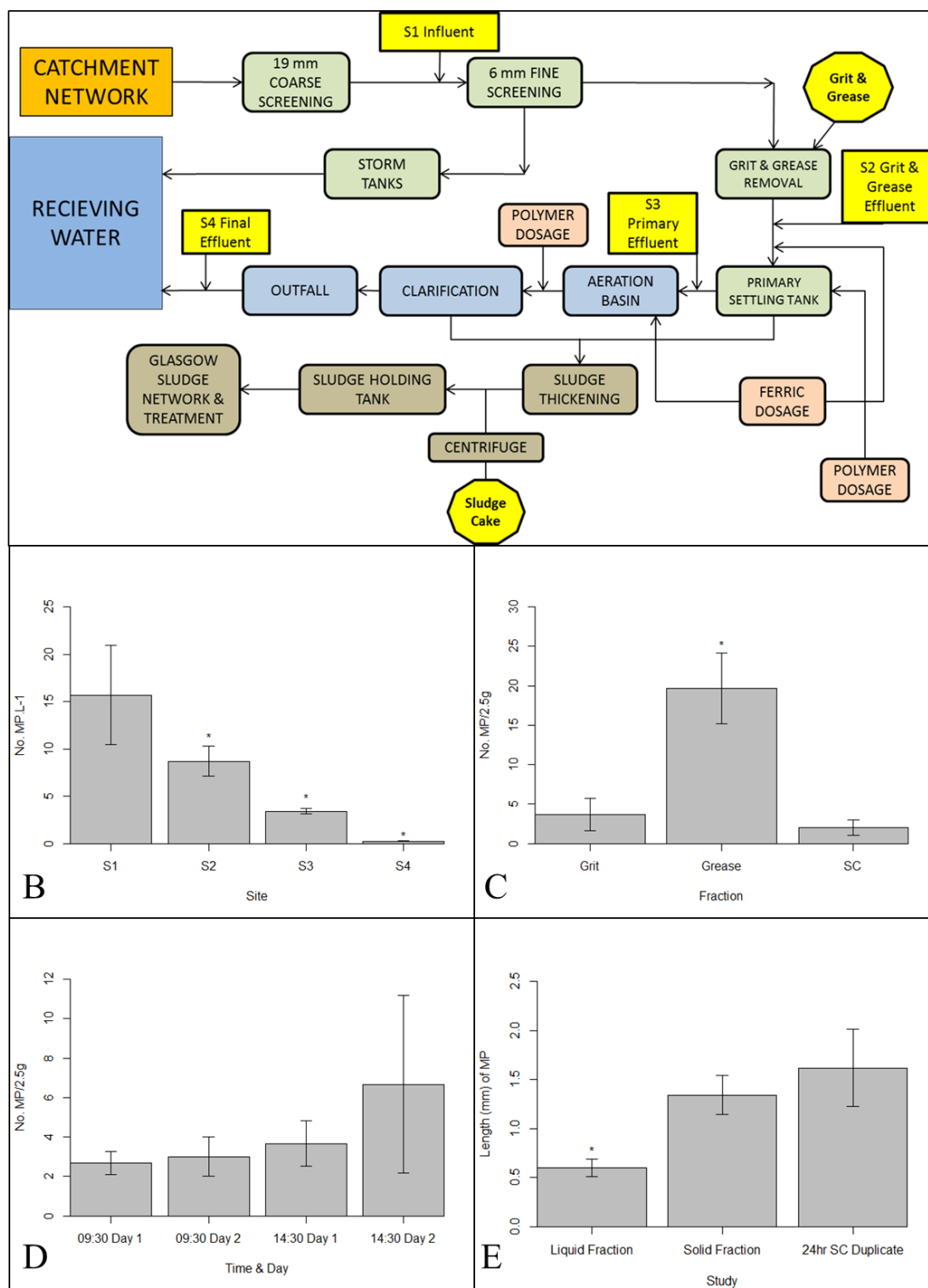


Figure 2.1. (A) Diagram of WwTW showing the location of the liquid fraction sampling sites (S1-4) where: S1 = Influent, S2 = Grit & grease effluent, S3 = Primary effluent, S4 = Final effluent. Sludge cake samples were taken from the same area for both the 24 hr SC duplicate comparison

and the comparison between grit and grease. (B) Barplot of the number of microplastic (MP). L⁻¹ at each liquid fraction site sampled (S1-4), (error bars = standard deviation, * = significance <0.05). (C) Barplot of the number of MP/2.5g from solid fraction comparison (error bars = standard deviation, * = significance <0.05). (D) Barplot of the number of MP/2.5g sample of 24hr SC duplicate (error bars = standard deviation). (E) Barplot of mean length of microplastic (mm) from each study (Liquid Fraction, Solid Fraction & 24hr SC duplicate) conducted (error bars = standard deviation, * = significance <0.05).

Table 2.1. Average number of MP Released at each sampling site per day and per year with percentage removal rates based on average outflow of 260,954 m³/Day. S1 = Influent, S2 = Grit & grease effluent, S3 = Primary effluent, S4 = Final effluent.

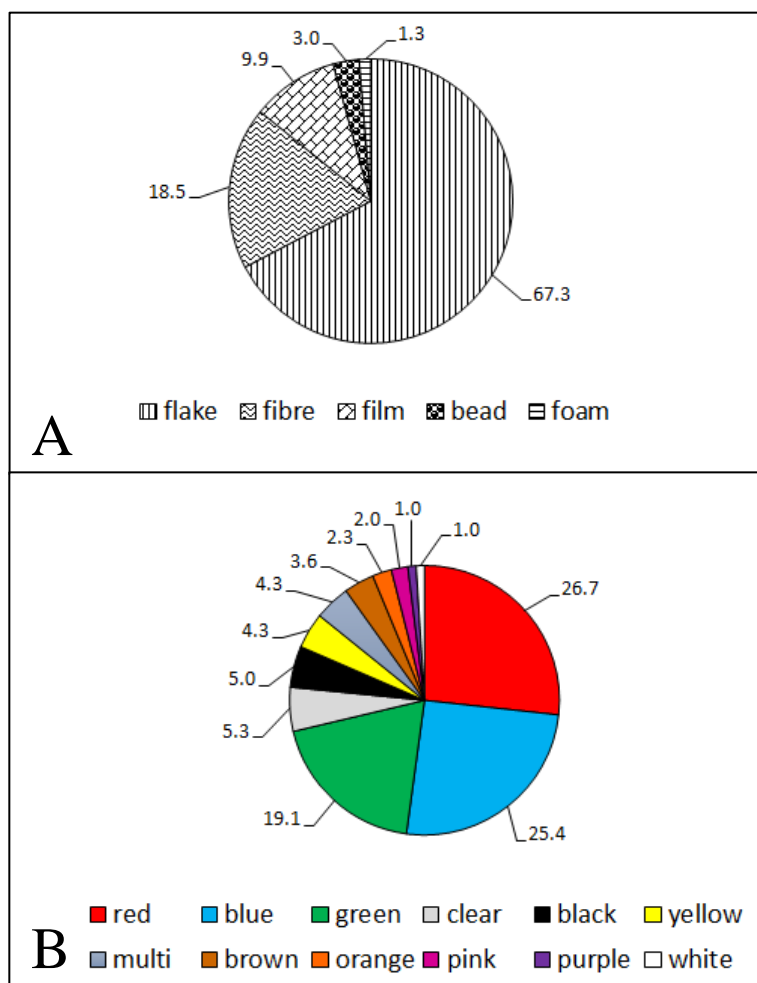
Site	MP. L ⁻¹	Million MP/Day	Million MP/Year	% Removal
S1	15.70 (±5.23)	4,097 (±1,365)	1,495,397 (±498,395)	0.00
S2	8.70 (±1.56)	2,270 (±406)	828,659 (±148,171)	44.59
S3	3.40 (±0.28)	887 (±74)	323,844 (±26,940)	78.34
S4	0.25 (±0.04)	65 (±11)	23,812 (±4,041)	98.41

1407 **Table 2.2.** The microplastics found in the liquid fraction (S1 = Influent, S2 = Grit & grease effluent, S3 = Primary effluent, S4 = Final
1408 effluent.), solid fraction and 24hr SC duplicate as a percentage of the total plastic found (PET = polyethylenterephthalat, PS acrylic =
1409 polystyrene acrylic, PV Acrylate = polyvinyl acrylate, PVA = polyvinyl acetate, PVC = polyvinyl chloride, PVE = polyvinyl ethelene).

Polymer	Liquid Fraction (303 MP)				Solid Fraction (79 MP)			24hr SC duplicate (48 MP)			
	S1	S2	S3	S4	Grit	Grease	SC	Day 1		Day 2	
								09:30:00	14:30:00	09:30:00	14:30:00
Acrylic	8.3	12.6	5.9	12.0	0.0	6.8	16.7	12.5	18.2	33.3	15.0
Alkyd	28.7	17.2	20.6	8.0	54.6	13.6	16.7	0.0	27.3	33.3	5.0
PET	3.8	12.6	2.9	4.0	0.0	13.6	0.0	0.0	0.0	0.0	0.0
Polyamide	4.5	2.3	14.7	20.0	9.1	0.0	0.0	0.0	0.0	0.0	10.0
Polyaryl ether	0.0	1.2	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0
Polyester	10.8	13.8	29.4	28.0	27.3	23.7	16.7	25.0	36.4	11.1	30.0
Polyethylene	4.5	1.2	14.7	4.0	0.0	32.2	33.3	0.0	9.1	0.0	5.0
Polypropylene	2.6	1.2	5.9	12.0	0.0	5.1	0.0	12.5	0.0	22.2	20.0
Polystyrene	2.6	17.2	5.9	4.0	9.1	1.7	16.7	37.5	0.0	0.0	10.0
Polyurethane	8.9	8.1	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0
Polyvinylfluride	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PS Acrylic	19.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PV Acrylate	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PVA	3.2	10.3	0.0	4.0	0.0	1.7	0.0	12.5	9.1	0.0	0.0
PVC	1.3	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PVE	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

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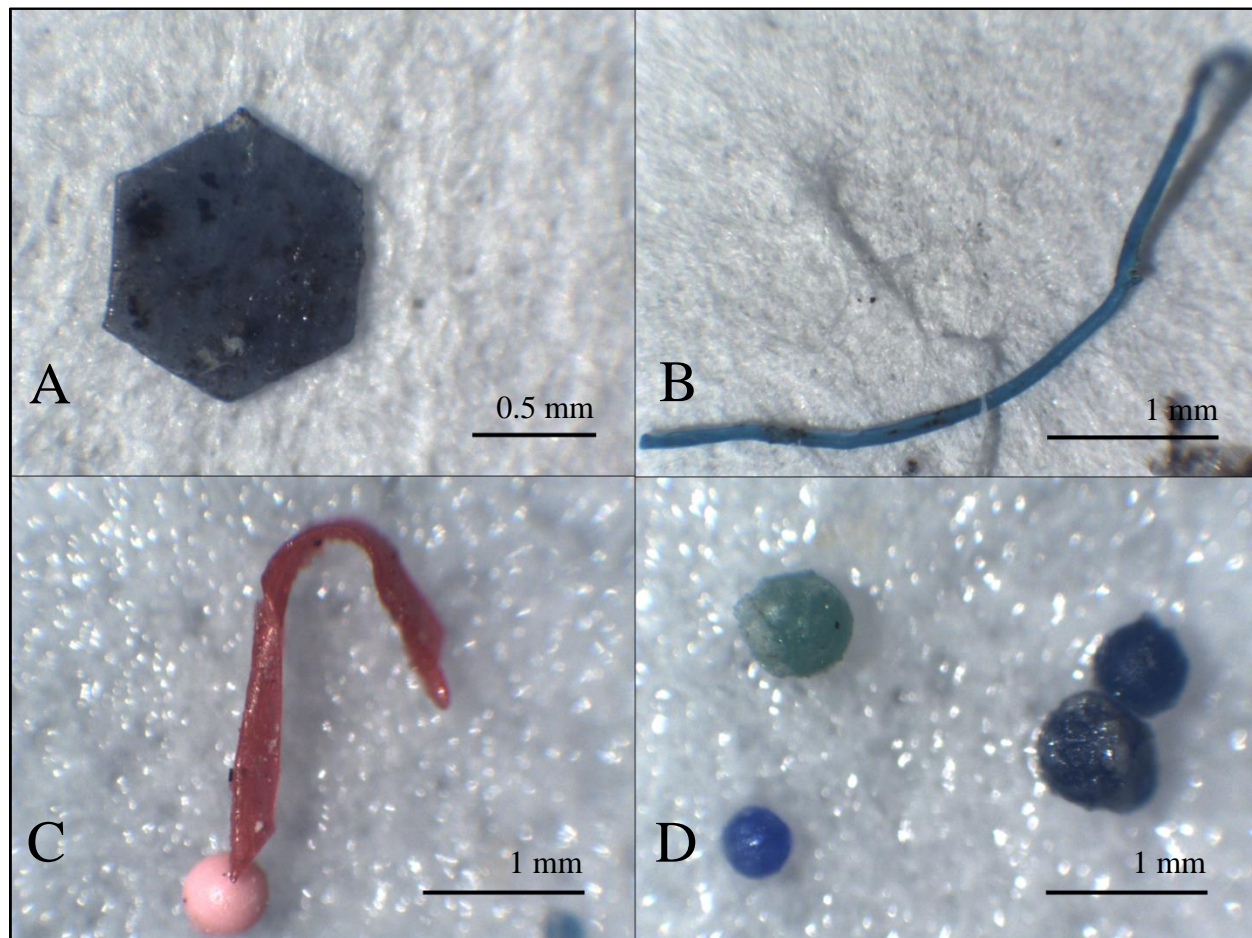
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1413 **Figure 2.2.** Pie charts of the different types (A) and colour (B) of microplastic found in all of the
 1414 liquid fraction samples examined from a secondary WwTW. Results shown as a percentage.

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1417 **Figure 2.3.** Photos of microplastics found in the solid fraction taken from a secondary WwTW.
1418 (A) Alkyd fragment taken from the sludge cake (SC) fraction. (B) Polypropylene fibre taken from
1419 the grit fraction. (C) A single polyethylene microbead and red PET fragment taken from the grease
1420 fraction. (D) 4 polyethylene microbeads extracted from the grease fraction.

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Supporting Information

This information is available free of charge via the Internet at <http://pubs.acs.org>.

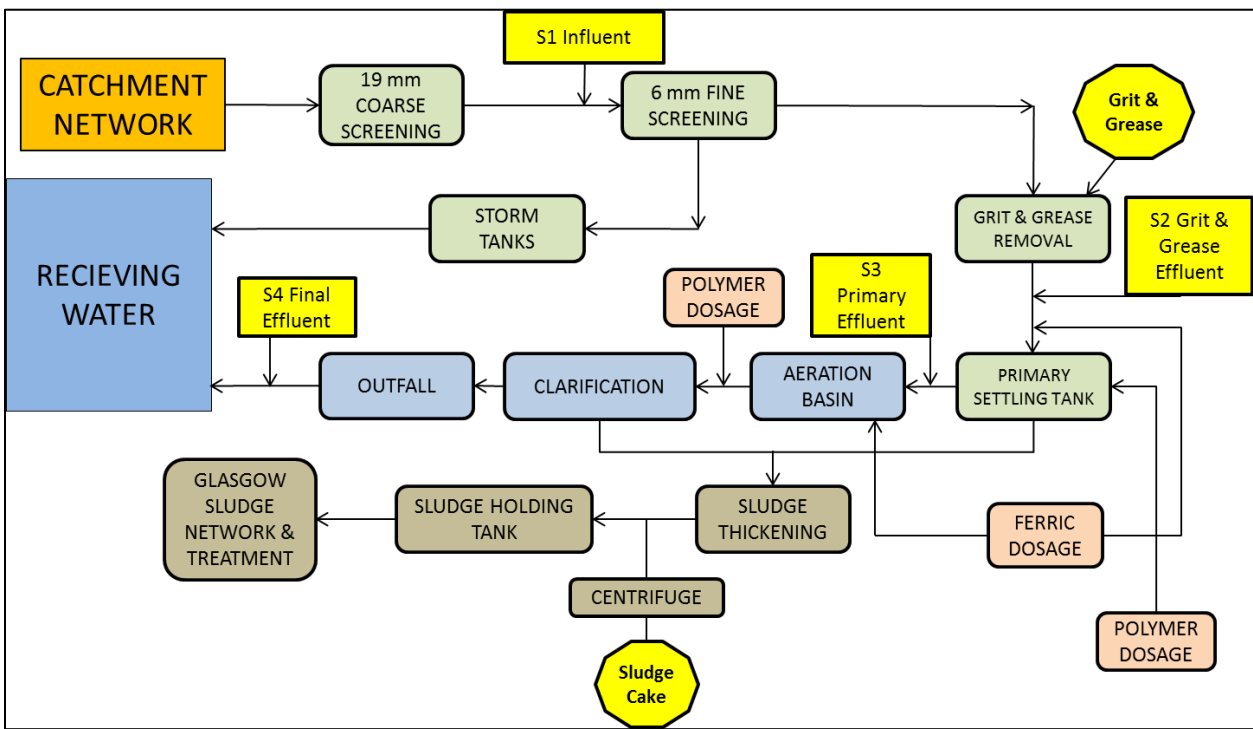


Figure S2.1 Diagram of WwTW showing the location of the sampling sites (yellow). Effluent (S1-4) sampling sites were: S1 = influent, S2 = grit & grease effluent, S3 = primary effluent, S4 = final effluent. The solid fraction consists of Grit & Grease and Sludge Cake and the temporal study consists of Sludge Cake.

Catchment Network (orange): This is the area served by the WwTW.

Pre-treatment & Primary Treatment (green): Course and fine screening removes larger debris such as branches, rocks, cans, etc. Grit & Grease removal allows for the settlement of heavier material such as sand and stones (grit) while grease is collected on the surface by skimmers. The primary settling tank allows debris to settle at the bottom of the tank while debris that floats to the surface is skimmed off. Storm tanks are used to hold wastewater when the incoming volume exceeds the treatable volume. If the storm tanks are full wastewater is then released untreated into the receiving water.

1442 Biological Treatment (blue): The aeration basin promotes biological oxidation of the wastewater.
1443 Clarification allows for the additional settlement of debris. The outfall is where the final treated
1444 effluent is released, with the receiving water being the final destination for the treated effluent.

1445 Sludge Treatment (brown): Sludge Thickening involves increasing the solid content of the sludge
1446 by removing some of the liquid portion. A centrifuge is used to reduce the water content of the
1447 sludge. Sludge holding tanks are used to store the sludge until it can be removed. Glasgow Sludge
1448 Network & Treatment involves incineration of the sludge in a waste to energy scheme.

1449 Flocculation Agents (pink): Ferric & polymer dosage: Chemicals such as ferric sulphate and
1450 polyacrylamide are used in the treatment process in order to cause suspended particulate matter to
1451 aggregate together.

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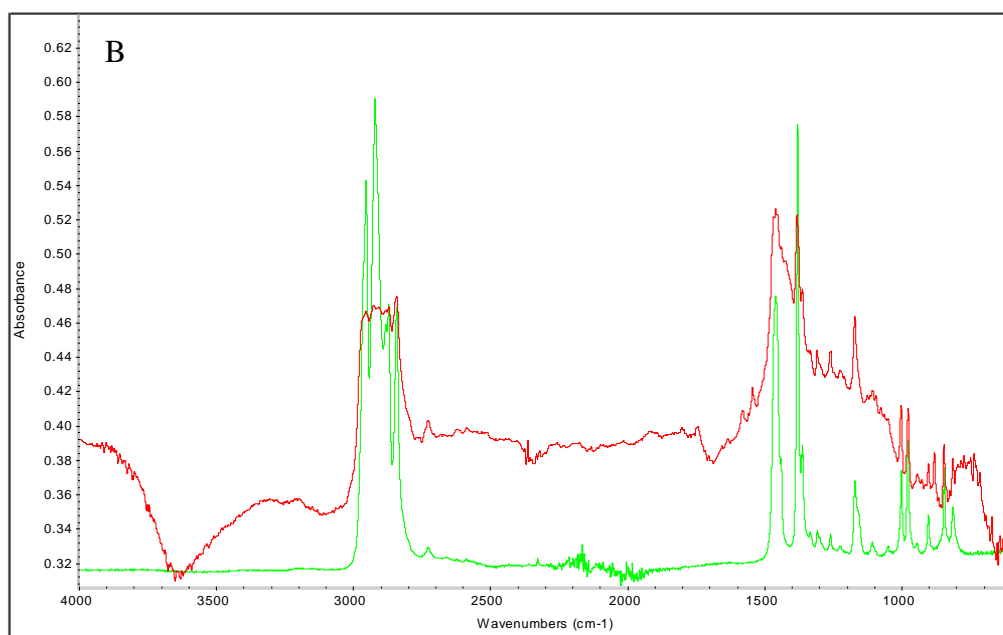
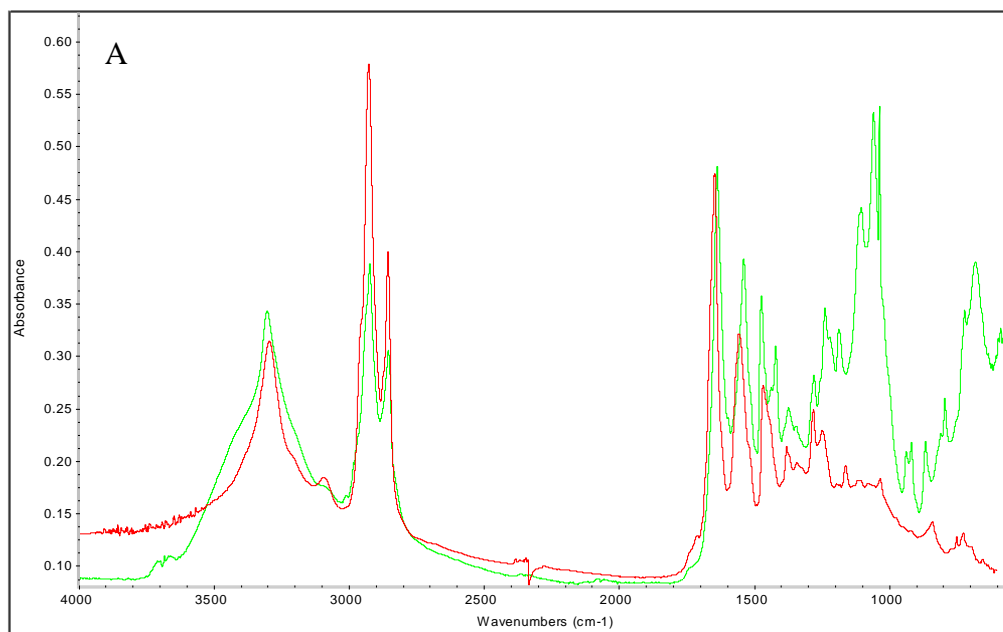
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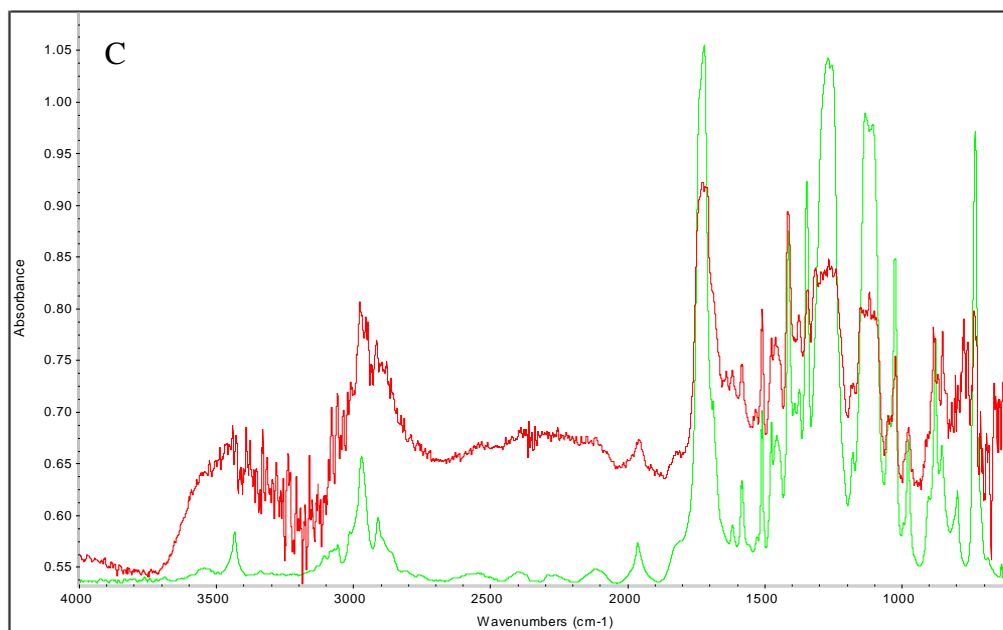


Figure S2.2. The comparison of FT-IR spectra found from a polymer library (green) with samples of the same polymer type found in the environment (red). (A) Blue polyamide flake sample spectra extracted from grit (red) and reference polyamide spectra (green). (B) Blue polypropylene fiber sample spectra extracted from pre-treatment & primary treatment (S3) (red) and polypropylene reference spectra (green). (C) Black polyethylene terephthalate (PET) fiber sample spectra extracted from the influent (S1) (red) and PET reference spectra (green).



Figure S2.3. (A) Photograph of cotton fibers collected on the forensic tapings undertaken as part of the contamination mitigation measures used in this study, magnification x 40 (B) A close up of a cotton fiber showing the distinctive ribbon like appearance making it easier to identify using light microscopy, magnification x 400

Chapter 3

Aquaculture as a potential source of microplastic in the marine environment.

Abstract

As microplastics are proving to be ubiquitous in the marine environment, it is vital that sources of microplastic pollution are identified to prevent or reduce their release into marine waters. The aquaculture industry uses synthetic netting and line to contain cultured fish and to act as a substrate for the growth of shellfish. This synthetic material is kept in the environment where it can start to degrade and fragment potentially releasing microplastic into the environment. Wild *Mytilus edulis* (blue mussel) and sediment were sampled from areas around fish aquaculture sites as well as the gastrointestinal tracts of cultured *Hippoglossus hippoglossus* (Atlantic halibut) and *Oncorhynchus mykiss* (rainbow trout) and were analysed for the presence of microplastic. Using the enzyme trypsin, *M. edulis* tissue was digested, filtered and any microplastic present were extracted and identified. Wild *M. edulis* were found to contain microplastic at all sites investigated with 47.92% containing microplastic and a mean number of microplastic ingested of 0.14 ± 0.19 particles per g^{-1} wet weight (w.w.), with no significant differences between sites. Microplastic was identified in 60% of cultured *H. hippoglossus* with a mean number of 0.009 ± 0.009 particles per g^{-1} w.w. ingested, no microplastic was found in the *O. mykiss* or any of the sediment samples taken around the aquaculture sites. The microplastics identified in *M. edulis* were primarily blue (94.29%) polyamide (88.6%) fibres (100%). Microplastics identified in *H. hippoglossus* samples were also consisted of mainly blue (85.7%) polyamide (71.4%) fibres (100%) and likely shared a similar source with infrared spectra of ingested microplastic and rope and netting samples being similar.

Keywords: Microplastic, Aquaculture, *Hippoglossus hippoglossus* (Atlantic halibut), *Mytilus edulis* (Blue mussel), Sediment.

3.1. Introduction

The issue of plastic pollution has been given considerable attention for a number of decades (Wilber, 1987). Many marine mammals (Laist, 1987 Laist, 1997), birds (Azzarello & Van Vleet, 1987) and turtles (Mrosovsky, 2009) have been widely reported to being negatively affected by plastic pollution in the wild. Plastic is light weight, durable, cheap and produced in vast quantities (Derraik, 2002, Plastics Europe 2014). It is for these reasons that plastic pollution has become such a major threat to the environment however attention has mainly been focused on large plastic debris. The issue of much smaller pieces of plastic known as microplastics has been gaining increasing attention (Thompson et al., 2004). Microplastics are pieces of plastic < 5 mm (Arthur et al., 2009) and can be separated into two separate types primary and secondary microplastics. Primary microplastics are designed to be of a microscopic size such as microbeads found in personal care products as an abrasive material (Napper et al., 2015). Secondary microplastics are formed through the degradation of larger plastic material through exposure to environmental stressors such as UV radiation and the mechanical stressors of wind and wave action (Singh & Sharma, 2008).

Sampling of shorelines, oceans and lakes has shown that microplastics are ubiquitous in the environment (Browne et al., 2011, Eriksen et al., 2013a, Lusher et al., 2014). Microplastic pollution has been increasing in the marine environment with a variety of species observed to have ingested microplastic in the wild (Lusher et al., 2013, Sanchez et al., 2014, Desforges et al., 2015, Devriese et al., 2015 Van Cauwenberghe et al., 2015) and many species have been proven to be capable of ingesting microplastic in laboratory exposures (Cole et al., 2013, Watts et al., 2014, Cole et al., 2015). Sources of microplastic include wastewater treatment works releasing microplastic in treated effluent (Browne et al., 2011 Magnusson & Norén, 2014, Murphy et al., 2016) as well as plastic being blown offshore from land based sources (Jambeck et al., 2015) and breaking down once it enters the environment (Singh & Sharma, 2008). Commercial fishing is also a source of microplastic in the environment as much of the fishing line and netting is made of synthetic material (Andrady, 2011, Law & Thompson, 2014). The aquaculture industry uses similar material to contain various fish species and to act as a substrate for the growth of shellfish, however little research has been carried out to determine if aquaculture is a significant source of microplastics in the environment and there has been no research undertaken to determine if

cultured fish are ingesting microplastic. Polystyrene floats used in aquaculture in South Korea were thought to fragment and pollute shoreline sediment (Heo et al., 2013).

Cultured mussels have been investigated previously for the presence of microplastics (De Witte et al., 2014, Mathalon & Hill 2014, Van Cauwenberghe & Janssen, 2014, Li et al., 2016). An average of 0.36 ± 0.07 particles g^{-1} in *Mytilus edulis* and 0.47 ± 0.16 particles g^{-1} in *Crassostrea gigas* was observed in North Sea coastal locations (Van Cauwenberghe & Janssen, 2014). However, this study utilised acid digestion (69% nitric acid) to breakdown organic matter and filter out any microplastics present which could potentially degrade microplastics that are present in the samples (Claessens et al., 2013). A study in Nova Scotia, Canada investigated microplastic occurrence in wild and cultured *M. edulis* finding significantly higher amounts of microfibrils in cultured *M. edulis* (average of 375 per 5 mussels) then in wild *M. edulis* (average of 170 per 5 mussels) (Mathalon & Hill 2014), however visual identification was used which has the potential to overestimate the amount of microplastic present (Hidalgo-Ruz et al., 2012, Rocha-Santos & Duarte 2015). Enzymatic digestion has been shown to be an efficient method of separating microplastic from organic matter while at the same time avoiding degradation of any potential microplastics present (Catarino et al., 2016, Courtene-Jones et al., 2017). Wild *M. edulis* sampled from the west coast of Scotland that were investigated for microplastic ingestion using enzymatic digestion were found to contain 1.05 ± 0.66 to 4.44 ± 3.03 microplastic particles g^{-1} wet weight mussel tissue (Courtene-Jones et al., 2017), which is higher than what was found in cultured *M. edulis* (Van Cauwenberghe & Janssen, 2014), these differences in the amount of microplastic present could be attributed to the different treatment techniques used to extract the microplastics from the sample tissue.

Aquaculture involves the use of synthetic netting to contain fish and synthetic line to act as a substrate for the growth of shellfish. As this synthetic netting and line is exposed to the environment it could potentially be breaking down and ingested by cultured as well as wild organisms. In the current study aquaculture was investigated as a source of microplastic by examining (i) wild *M. edulis* located nearby aquaculture sites for microplastic ingestion using enzymatic digestion (ii) the gastrointestinal tracts of two cultured fish species for ingested microplastic (iii) sediments sampled nearby aquaculture sites for the presence of microplastics.

3.2. Materials & Methods

3.2.1 Site Description

This study was carried out in Loch Melfort on the west coast of Scotland (Figure 3.1). Loch Melfort is a designated shellfish and fish growing site with the fish growing area being the more extensive of the two. The site is classified as having good overall status based on the Water Framework directive (WFD) (Directive 2000/60/EC) coastal waters classification system used in Scotland. The mouth of Loch Melfort faces south west but is sheltered from prevailing winds by the islands of Luing and Shuna (SEPA, 2011). The wind direction is primarily from the southeast and Northwest and the Loch has a maximum depth of 73m. To the northwest of Loch Melfort is the Seil Sound, Argyll which is also a designated shellfish aquaculture area containing seven active shellfish sites (Figure 3.1). There are a number of active aquaculture operations in Loch Melfort, with six active fish sites and two active shellfish sites as well as a secondary wastewater treatment works serving a population equivalent of 500 (Marine Scotland, 2017). Loch Melfort also contains a marina with 250 berths.

3.2.2 Sample Organisms

Wild *Mytilus edulis* (n = 48) and cultured *Hippoglossus hippoglossus* (Atlantic halibut) (n = 10), *Oncorhynchus mykiss* (rainbow trout) (n = 6) and sediment (4 sites) were sampled from around aquaculture sites and their surroundings (Figure 3.1). Due to the differences in sample composition and weight it was necessary to employ a variety of microplastic extraction protocols that best suited the individual samples examined.

3.2.2.1 Wild *Mytilus edulis*

Wild *M. edulis* were collected from four sites on the west coast of Scotland adjacent to an aquaculture farm (from floating pontoons approx. 5 metres from the submerged aquaculture nets), a mussel aquaculture farm (from the shore about 10 metres from mussel long line) and two marina pontoons (inshore and at the mouth of the marina) roughly 6.5 km south of the aquaculture sites sampled and 2 km East of another fish site (Figure 3.1). Samples were transported to the laboratory in sealed aluminium containers where they were frozen at -20°C until processed. Enzymatic digestion was used to separate the microplastics from the soft tissue of *M. edulis* following the procedure described by Courtene-Jones et al., (2017). Briefly, samples were removed from the

freezer, weighed and measured (length, width, height) using callipers (Table 3.1). The samples were then allowed to defrost over ice before having their visceral mass removed using a scalpel and weighed. The samples were quartered under a dissection microscope and placed in a beaker that contained 20 ml of a 0.3125% trypsin solution. The beaker was then left on a hot plate with a magnetic stirrer at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and left to stir for 30 min. After which the mixture is poured through 80 μm mesh, the mesh is then examined under a dissection microscope. Using fine forceps any potential microplastics are carefully removed and placed on a clean 30 mm filter paper and sealed in a clean petri dish until identification.

3.2.2.2 Cultured Fish Species

The gastrointestinal tracts of cultured *H. hippoglossus* and *O. mykiss* were dissected on site in the fish farm and stored at -20°C and transported frozen back to the laboratory. In the laboratory, samples were defrosted over ice and using clean scissors and forceps the gastrointestinal tracts were carefully dissected and examined under a dissection microscope (Lusher et al., 2013). After examining the tissue thoroughly, the contents were washed with ddH₂O and re-examined to dislodge and clean any potential macroplastic or microplastic that had been obscured by the gastrointestinal tissue or contents. Any non-prey item, which is any item that did not appear to be part of the natural diet of the sample or appeared to be synthetic in nature, was removed and placed on a clean filter paper and sealed in a plastic petri dish until identification.

3.2.2.3 Sediment

Sediment samples were collected from a boat using a day grab at two fish aquaculture sites, Kames East Cage and Shuna (Figure 3.1) with two sediment samples taken from each location. The first sample was taken adjacent to fish cages while the second was taken 50 m from the cages. The sediment was separated into size classes of 400, 200, 100, 80 & $< 80 \mu\text{m}$ by passing them through stainless steel sieves and 50 mg sub samples of each size class were taken in triplicate and examined under a dissection microscope. Any potential microplastics were removed using fine forceps and placed on a clean 30 mm filter paper in a sealed petri dish until they were identified.

3.2.3 Contamination Mitigation

Sample analysis was undertaken in a clean laboratory following a strict contamination protocol (Murphy et al., 2016). Briefly, all equipment used was cleaned and examined under a dissection

microscope for contamination, clean white cotton lab coats were worn at all times and all work surfaces were wiped down with 70% ethanol three times prior to work commencing. The tape lifting techniques used in forensic science laboratories to check laboratory benches for fibre and particle contamination was used in this study (Wheeler & Stancilffe, 1998). To take a taping, a piece of tape measuring 5 cm × 5 cm was randomly placed three times on the work surface after it was cleaned. After all lab work was completed, another taping was taken and was also placed on a sheet of acetate. The tapings were then examined under a microscope for identification. Tapings were carried out before and after all sample analysis. Atmospheric microplastic contamination was determined by leaving clean filters in petri dishes out for the duration of any sample analysis to collect any atmospheric microplastic that may be present.

3.2.4 Identification

All potential microplastics found were examined under a dissection microscope, described by their morphology (fibre, bead, flake, etc.) and colour, photographed and then positively identified using Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR). A Perkin Elmer Spectrum One ATR-FTIR (manufactured in Llantrisant, United Kingdom) with radiation set from 600 – 4000 cm⁻¹ was used allowing for the identification of chemical bonds present in the samples and also giving a characteristic signal in the “fingerprint” region. Using this technique and with the aid of a library of reference spectra polymers could be positively identified (Murphy et al., 2016).

3.2.5 Statistics

All statistics were carried in R-studio version 3.2.2. Fulton’s condition factor (CF) $K = 100 \times \frac{W}{L^3}$ was calculated for *M. edulis*. Comparisons between *M. edulis* sites were based on the number of microplastics found per g⁻¹ of *M. edulis* wet weight (w.w.). Significant difference between *M. edulis* sites were tested using the non-parametric Kruskal-Wallis test. Pearson moment correlations were carried out on the number of microplastics per g⁻¹ w.w. and mussel CF. A correlation was also carried out between the number of microplastic items per g⁻¹ w.w. ingested by *M. edulis* and the distance from aquaculture activity.

3.3. Results

Microplastics were identified at each site where *M. edulis* were sampled with 47.92% of all individuals examined containing microplastic (Figure 3.2A). *M. edulis* collected 10m from the mussel longline (10m MLL) had the lowest amount of ingestion at 41.67% (Figure 3.2A) while all other sites had the same amount of ingestion at 50%. In total 35 particles were identified as microplastic from 23 individuals with a mean number of 0.14 ± 0.19 microplastic particles per g⁻¹ (w.w.) ingested. There was no significant difference in the number of microplastic particles per g⁻¹ w.w. found in *M. edulis* between sites ($p = 0.74$) (Figure 3.2B) or significant correlation between *M. edulis* CF and the number of microplastic items per g⁻¹ w.w. ($p = 0.35$) (Table 3.1). The mean size of microplastic found in the *M. edulis* samples were 1.66 ± 0.91 mm. Polyamide was found in all *M. edulis* sites and represented 88.6% of microplastics identified, followed by PET (8.6%) and polyethylene (2.9%) (Figure 3.3A). *M. edulis* microplastics were almost entirely blue (94.29%) in colour followed by black (2.86%) and green (2.86%) (Figure 3.3B) and consisted solely of fibres. There was no significant correlation between the number of microplastic items per g⁻¹ w.w. ingested by *M. edulis* and the distance from aquaculture activity ($p = 0.847$).

Microplastics were found in 60% of *H. hippoglossus* samples with 7 particles identified from 6 individuals or 0.009 ± 0.009 per g⁻¹ w.w.. These microplastics were 2.99 ± 2.13 mm in length and consisted primarily of polyamide (71.4%), acrylic (14.3%) and polyethylene terephthalate (PET) (14.3%) (Figure 3.4). *H. hippoglossus* microplastics were blue (85.7%) and black (14.3%) in colour (Figure 3.4) and consisted entirely of fibres. No microplastic was found in any of the *O. mykiss* samples.

The filters that were left out to collect any atmospheric microplastics throughout the duration of the study yielded 5 blue fibres with a mean length of 4.51 ± 1.72 mm. Three of these fibres were identified as cellulose, while two produced spectra that were too poor to successfully identify.

3.4. Discussion

This study shows that microplastics are present in wild *M. edulis* sampled from nearby aquaculture sites and in the gastrointestinal tract of the cultured demersal flatfish species *H. hippoglossus*. The microplastics identified were primarily blue polyamide fibres (Figure 3.3A & B) although other polymers were identified such as PET (Figure 3.3A) but in much lower quantities. Due to the similarity in polymer composition and morphology of the microplastics identified in the wild *M. edulis* and cultured *H. hippoglossus* it is highly likely that they originate from the same source. The wild *M. edulis* sampled from a marina approximately 2.0 km from the aquaculture sites had similar amounts of microplastic present per g⁻¹ w.w. to the *M. edulis* sampled from 5 m KFF & 10 m MLL aquaculture sites (Figure 3.2A & B). The aquaculture sites are located in the sheltered coastal waters of the west coast of Scotland in an area with little pollution making it ideal for the culturing of fish and shellfish. It is unlikely that there are significant amounts of microplastic entering this area from either land based source, however there is a secondary waste water treatment plant located around 9 km from the marina sampled with a population equivalent of 500.

Previous studies carried out on the west coast of Scotland examined microplastic ingestion in *M. edulis* finding 97% of the *M. edulis* examined had ingested microplastic (Courtene-Jones et al., 2017) and 84.1% of *Nephrops norvegicus* sampled contained microplastic (Welden & Cowie, 2016). These previous studies show considerably higher microplastic ingestion than the 47.92% in *M. edulis* observed in the current study. This is particularly interesting when compared to Courtene-Jones et al., (2017) which examined the same species and implemented the same enzyme digestion and contamination protocols as the current study but found 1.05 ± 0.66 (min) to 4.44 ± 3.03 (max) microplastic particles per g⁻¹ w.w. *M. edulis* tissue. The mean number of microplastic particles found in the current study was 0.14 ± 0.19 per g⁻¹ w.w. which is considerably lower than the minimum found in Courtene-Jones et al., (2017). It is likely the differences in microplastic ingestion are due to the different areas sampled, the time of year the samples were collected or proximity to potential sources of microplastic rather than being influenced by differences in methodology. The current study sampled *M. edulis* around aquaculture sites which are normally found in pristine marine waters to ensure a quality product is produced while Courtene-Jones et al., (2017) sampled *M. edulis* from an area with much higher maritime traffic and activity.

Courtene-Jones et al., (2017) identified a variety of potential sources such as WwTW effluent, commercial fishing activity as well as mussel and fish farms. Microplastics identified were primarily red and blue polyamide (72%) and fibres (86%) (Courtene-Jones et al., 2017) which is similar to the blue (94.3%) polyamide (88.6%) fibres (100%) found in this study (Figure 3.5). Polyamide has a range of application and is used widely in commercial fishing and aquaculture in nets and lines as well as the textile industry for the manufacturing of clothing.

While Van Cauwenberghe & Janssen, (2014) found on average 0.36 ± 0.07 particles per g⁻¹ w.w. in cultured mussels an attempt was made to identify the polymers however only synthetic pigments were successfully identified not the microplastic themselves. Studies have also been carried out previously in China (Li et al., 2016) and Belgium (De Witte et al., 2014) but these studies used acid digestion to breakdown organic matter which could inadvertently destroy microplastic that are present making comparisons difficult. The Belgian study reported microplastic amounts of between 0.26 to 0.51 fibres per g⁻¹ of *M. edulis* tissue (De Witte et al., 2014) which is similar to what was found in the current study while the Chinese study sampled 9 commercial bivalve species found an average of 2.1 to 10.5 items per g⁻¹ (Li et al., 2016). De Witte et al., (2014) only found fibres of various colours ranging in size from 200 µm to 1500 µm with identification was done visually while Li et al (2016) mostly found fibres and fragments and identified some of the microplastic as PET and polystyrene.

This study is the first to provide evidence that aquaculture reared fish are ingesting microplastic. Of the two species examined for microplastic ingestion only *H. hippoglossus* was found to have ingested microplastic with 60% of individuals containing blue (85.7%) polyamide (71.4%) fibres (100%) which is similar to the wild mussel samples. No microplastics were found in the *O. mykiss*. This may be due to the small number of *O. mykiss* (n = 6) examined but may also be influenced by the free swimming behaviour of these fish that are less likely to graze the nets.

In contrast to this behaviour, the flatfish *H. hippoglossus* sit at the bottom of the enclosure where they can graze the net and are therefore more likely to come into contact with microplastics. This species is also slow growing and will spend more time in the nets, potentially increasing the time of exposure to microplastics. Similar rates of ingestion have been observed in wild demersal fish taken from the English Channel (Lusher et al., 2013), where 51.5% of *Aspitrigla cuculus* (red gurnard) were found to contain plastic the majority of which was <5mm. Preliminary results from

a study conducted by the authors investigating the ingestion of microplastic by demersal flatfish sampled in nearby Scottish coastal waters found microplastic to be ingested by 39.1% of fish examined (submitted for publication). Which is lower than what was found in the *H. hippoglossus* examined in this study, this may indicate that cultured flatfish are ingesting microplastic at a higher rate than wild fish. Microplastic ingested by the wild demersal fish were also mainly polyamide (65.5%) but only 13.4% of the microplastics were blue in colour. The sample size of *H. hippoglossus* investigated in this study was quite small therefore greater sampling effort would be required to determine the ingestion rates of this cultured fish as well as the composition of microplastic ingested before an appropriate comparison can be made with wild fish in the area.

No microplastic was found in any of the sediment samples collected. Initially density separation was to be carried out on the sediment to extract any microplastic present (Quinn et al., 2017), however due to the silty sediment floating this was not feasible. It was then decided to separate out the microplastic into different size fractions and then take 50 mg subsamples of the sediment and manual sort through it to pick out any potential microplastics. The sediment samples did contain considerable amounts of debris however this debris mainly consisted of material with organic origins such as plant based material and calcium carbonate originating from the shells of animals. The lack of microplastic in the sediment nearby the aquaculture sites may be caused by any microplastic released being transported away from the cages before it can settle in nearby sediments or accumulating in areas not sampled. The microplastic will also be less dense than the seawater and will float if released although biofilms can form on the microplastic which can alter its buoyancy (Oberbeckmann et al., 2015).

More appropriate methods to extract microplastics from silty sediment will need to be developed as well as a greater sampling effort of the sediment located around the aquaculture cages to determine if microplastic is present or absent from these sites. Microplastic has been found to occur in sediments throughout the world (Browne et al., 2011) and was found in all *M. edulis* sites sampled so it is likely that microplastics are present in the area sampled but due sampling difficulties were not identified. Shore sampling was not possible in this location as it was a steeply shelving rocky shore with no sand or sediment present, which is one of the reasons that this location makes an ideal fish aquaculture site. Analysis of the surrounding waters for microplastics would also be of interest to determine the concentration of microplastics present in the water column or

surface waters but was not possible in the current study. Sampling of shorelines in an area where *M. edulis* were found to be ingesting microplastic were found to contain between 20 to 80 microplastics/10g of sediment while higher amounts of microfibrs in cultured *M. edulis* (average of 375 per 5 mussels) then in wild *M. edulis* (average of 170 per 5 mussels) were also found in the same area (Mathalon & Hill 2014).

The polyamide found in the current study may potentially originate from the aquaculture sites but may also be originating from recreational activity or land based inputs such as waste water effluent (Murphy et al., 2016), however it is difficult to determine the exact source of the microplastics once they enter the environment. Net & rope samples used in the Kames Fish farm aquaculture sites were collected and identified using FTIR and compared with the ingested microplastics (Figure 3.6). The resulting spectra of the polyamide netting and ingested polyamide (Figure 3.6A & B) and the PET rope and ingested PET (Figure 3.6C & D) were similar however it does not prove the netting and rope are the source. Net & rope samples used primarily for containing cultured fish consisted of polyamide, PET and polypropylene while general use ropes were found to consist of polypropylene. Although polyamide was the most abundant microplastic found in all samples and sites the polyamide used in Kames was black. Blue was by far the most commonly found colour of microplastic found in this study however only one of the net and rope samples identified was blue with both identified as polypropylene rope for general use. Polyamide ropes are used by kames fish farm for moorings and positioning of net. There are other active aquaculture sites that may be using blue polyamide netting or ropes which could be the source of this microplastic, however sample of these could not be acquired for identification.

There are a cluster of seven cultured shellfish sites near the WwTW approximately 9 km north of the marina pontoon sites as well as four active fish sites around Shuna Island (Figure 3.1). We were unable to collect rope sample from these sites, it is therefore not possible to determine if these were the source of the microplastic fibres. However, the mussel farms were observed to use blue ropes but it is unknown what polymer these ropes consisted of. Due to the uniformity of the microplastic found as well as the clean water of this area it is unlikely that there are significant land based sources of microplastic although there is a small secondary WwTW present (Figure 3.1).

WwTW have been proven to release microplastic in several locations throughout the world (Browne et al., 2011, Magnusson, & Norén, 2014, Murphy et al., 2016). The removal rates are normally quite high, for example a secondary WwTW in Scotland serving a population equivalent of 600,000 was found to release 0.25 microplastic particles per litre of treated effluent into the receiving water (Murphy et al., 2016). This study reported that 65 million microplastics per day or 108 microplastic particles per person per day were released within the final effluent. Using Murphy et al., (2016) figures that results in an estimated 54,000 microplastic particles released per day from the WWTW identified as a potential source in this study. This is unlikely to contribute significantly to the microplastic load of the area as this microplastic will become dispersed once it enters the receiving water. Murphy et al., (2016) reported microplastics to be on average 0.598mm (± 0.089) in size which is considerably smaller than the average size of microplastic found in the *M. edulis* (1.66 ± 0.91 mm) and *H. hippoglossus* (2.99 ± 2.13 mm). It would therefore seem unlikely that the microplastic recovered from the samples in this study are originating from this source. Although similar types of microplastic were identified in the final effluent such as polyester, polyamide, polypropylene and acrylic the morphology and colour varied considerably (Murphy et al., 2016). Due to the uniformity of the microplastic identified and the efficiency of wastewater treatment plants in the capture of microplastics (Murphy et al., 2016) it seems unlikely to be originating from this source. It is also unlikely that aquaculture activities would be carried out at this site if waste water inputs from this facility were significant. The marina located nearby may be a source of microplastics as recreational vessels would also use synthetic rope. However, the *M. edulis* collected by the marina did not have significantly higher amounts of microplastic than those sampled further away. It is therefore unlikely that the marina is a significant source of these microplastic fibres.

There was no contamination found on the atmospheric filters left out over the duration of the study. Although five blue fibres were found on the filter, three were identified as cellulose and two produced spectra that were too poor to interpret however they were similar to the cellulose fibres identified. The fibres were also considerably longer than ($4.51 \text{ mm} \pm 1.72$) than the *M. edulis* ($1.66 \text{ mm} \pm 0.91$) or *H. hippoglossus* ($2.99 \text{ mm} \pm 2.13$) microplastics. The tapings taken of the work area contained items similar in appearance to the cellulose fibres identified in the atmospheric filter and those identified in a previous study which used the same techniques (Murphy et al.,

2016). Based on the material on the filters and the tapings taken it is unlikely that any significant contamination occurred over the course of this study.

3.5. Conclusions

This study provides evidence that microplastic is being ingested by cultured flat fish and wild mussels growing nearby aquaculture sites. However, these concentrations are lower than those found in mussels further up the west coast of Scotland and in wild demersal fish from Scottish marine coastal waters. The Scottish aquaculture industry is expected to grow from £1.8 billion to £3.6 billion by 2030. This growth will invariable affect the marine environment, it is therefore important to understand the potential impact of this growth in order to minimise any negative effects. The potential release of microplastic from aquaculture activity is still not well understood. As continued growth in the aquaculture industry is projected to occur worldwide (FAO, 2014), it is of great importance that the potential contribution aquaculture activity has on the input of microplastic pollution in the marine environment is determined to prevent or mitigate any potential negative impacts.

Acknowledgments

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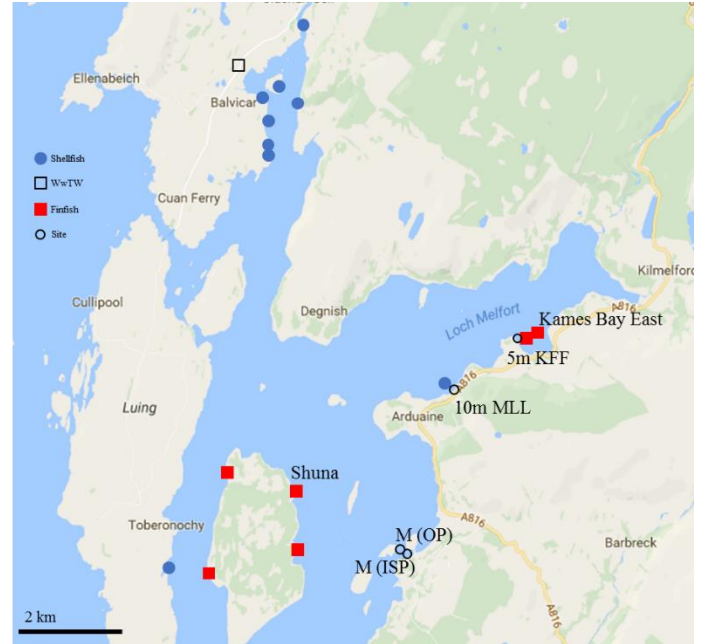


Figure 3.1. Map of Loch Melfort and the surrounding area showing sampling sites (black ring), shellfish aquaculture (blue circle), fish aquaculture (red square) and WwTW = Wastewater treatment works (black square). 10m MLL = 10m from mussel longline; 5m KFF = 5m from Kames Fish Farm; M (ISP) = Marina (inshore pontoon); M (OP) = Marina (offshore pontoon).

Table 3.1 *M. edulis* physiology data at each site sampled presented as the Mean (\pm Standard deviation (Stdev)). n = number; 10m MLL = 10m from mussel longline; 5m KFF = 5m from Kames Fish Farm; M (ISP) = Marina (inshore pontoon); M (OP) = Marina (offshore pontoon); CF = (Fulton's condition factor ($K = 100 \times \frac{W}{L^3}$)).

Site	n	Length (cm)	Width (cm)	Height (cm)	Total mass (g)	Visceral mass (g)	CF
10m MLL	12	6.26 (± 0.47)	2.58 (± 0.30)	3.18 (± 0.18)	22.04 (± 6.47)	6.77 (± 1.63)	8.84 (± 1.68)
5m KFF	12	6.13 (± 0.50)	2.23 (± 0.24)	2.95 (± 0.27)	13.54 (± 3.47)	5.94 (± 1.51)	5.84 (± 1.05)
M (ISP)	12	5.87 (± 0.53)	2.37 (± 0.25)	3.14 (± 0.24)	16.22 (± 4.19)	6.42 (± 2.06)	8.00 (± 1.43)
M (OP)	12	5.29 (± 0.74)	2.07 (± 0.30)	2.73 (± 0.24)	12.01 (± 4.09)	4.24 (± 1.39)	8.06 (± 1.39)

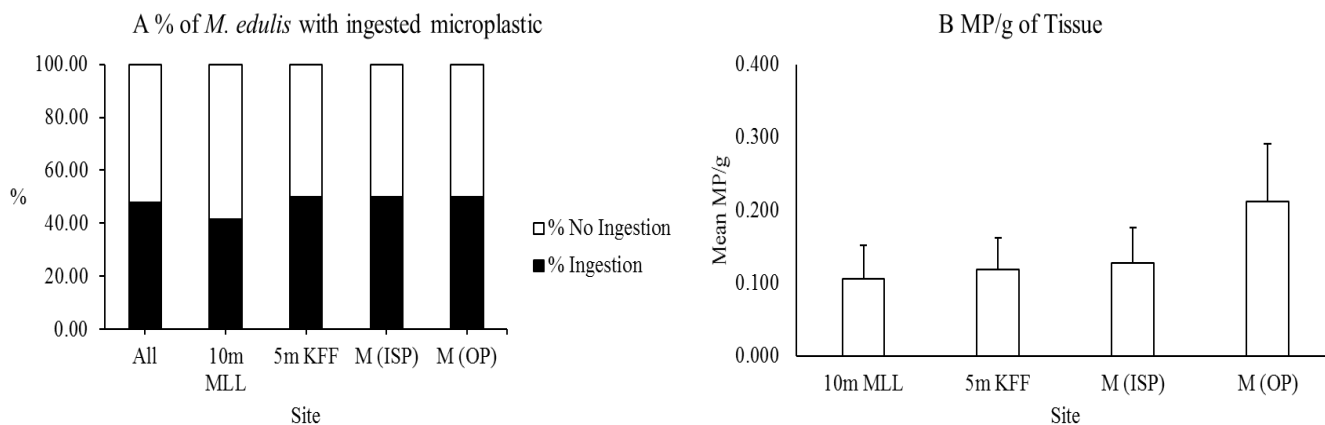


Figure 3.2. Barcharts showing (A) percentage of *M. edulis* with ingested microplastic at each site. (B) the mean number of microplastics (MP) found in *M. edulis* per g⁻¹ wet weight (w.w.) at each site, error bars = standard error of the mean. 10m MLL = 10m from mussel longline; 5m KFF = 5m from Kames Fish Farm; M (ISP) = Marina (inshore pontoon); M (OP) = Marina (offshore pontoon).

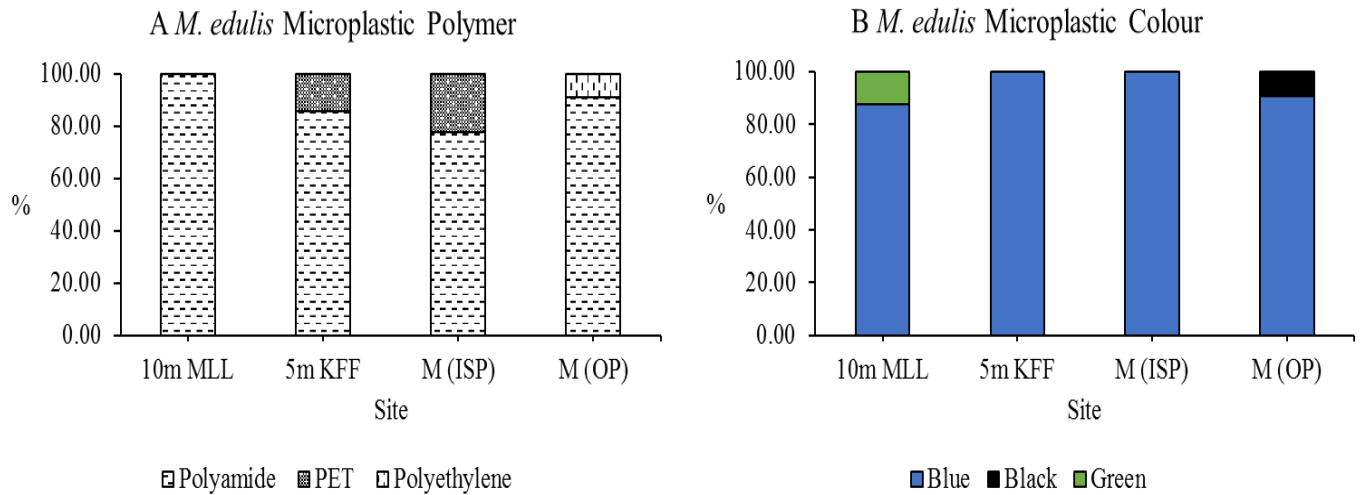


Figure 3.3. Barcharts showing (A) the percentage of each polymer type of the microplastics identified in *M. edulis* samples at each site. (B) the percentage of each colour of the microplastics identified in *M. edulis* samples at each site. PET = polyethylene terephthalate; 10m MLL = 10m from mussel longline; 5m KFF = 5m from Kames Fish Farm; M (ISP) = Marina (inshore pontoon); M (OP) = Marina (offshore pontoon).

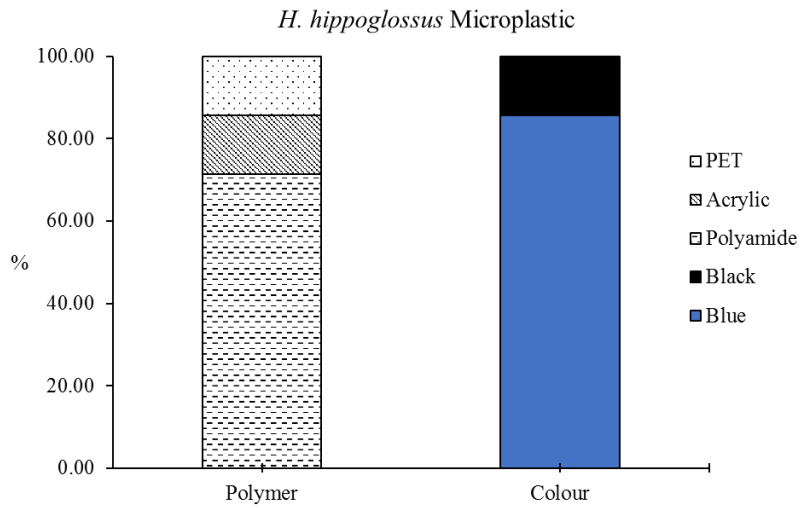


Figure 3.4. Barchart of the percentage of microplastics identified in *H. hippoglossus* based on polymer type and colour. PET = polyethylene terephthalate.

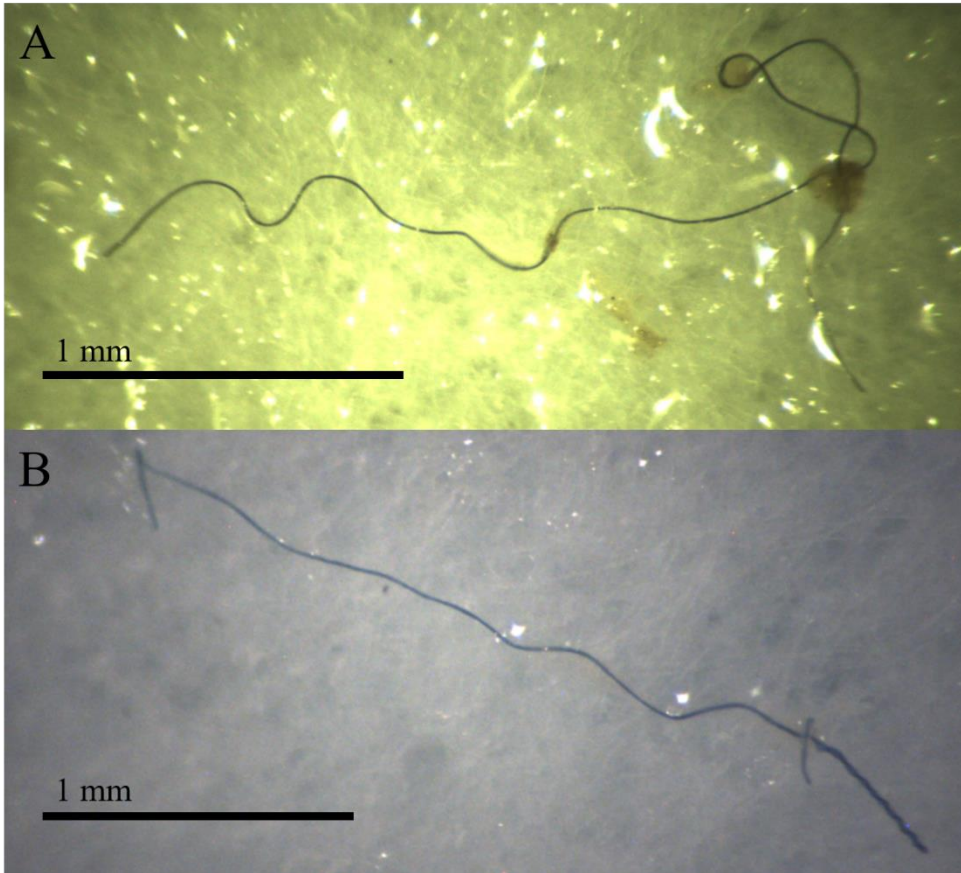
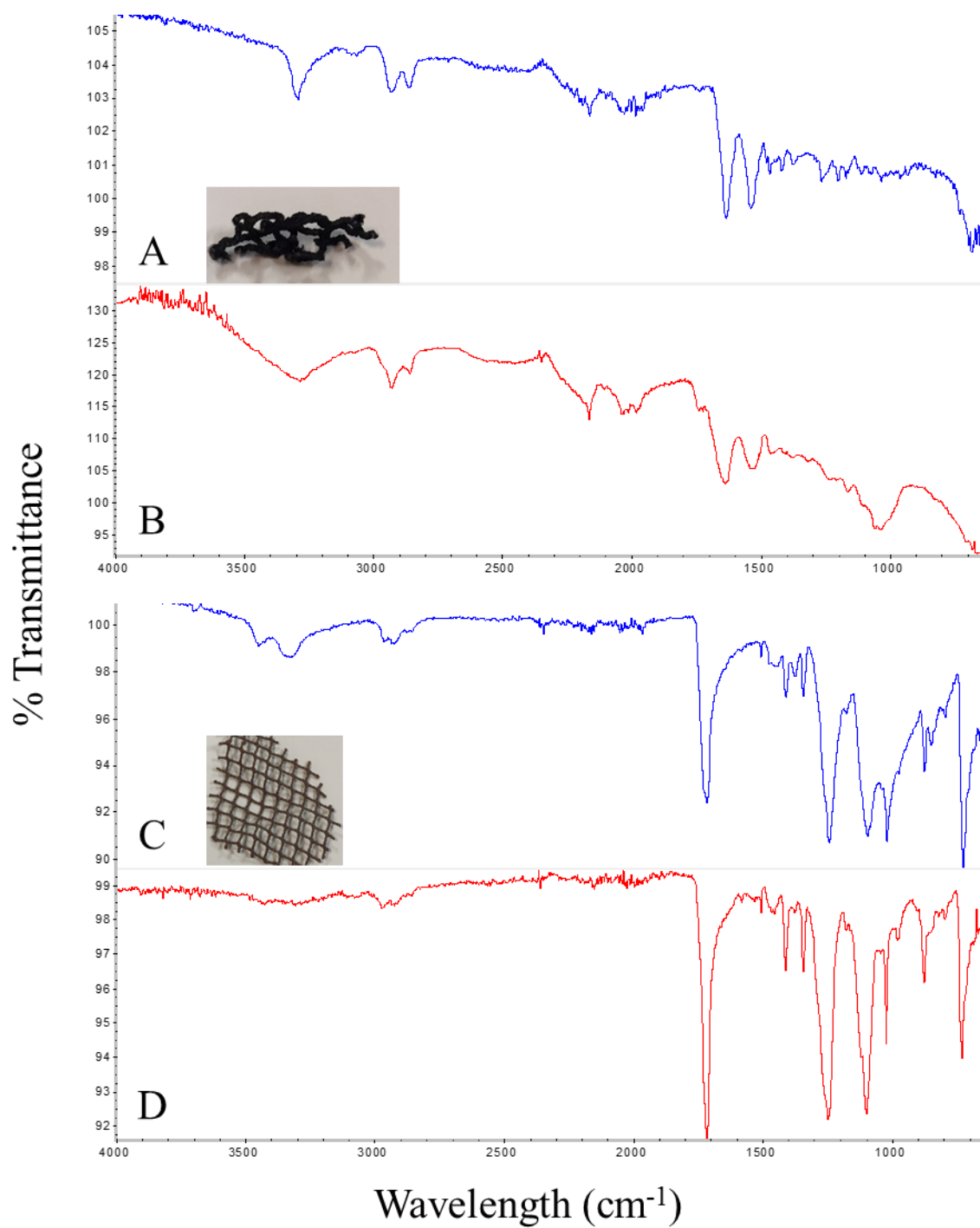


Figure 3.5. Photographs of microplastics identified from *Mytilus edulis* samples. (A) Blue polyamide fibre from wild mussels taken near mussel aquaculture facility. (B) Blue polyethylene terephthalate (PET) fibre from marina (inshore pontoon).



1972

1973 **Figure 3.6. Examples of spectra taken from (A) black polyamide netting used for cultured**

1974 **fish, (B) blue polyamide fibre extracted from a mussel, (C) brown/red polyethylene**

1975 **terephthalate (PET) net used to contain cultured fish and (D) blue PET fibre extracted from**

1976 **a mussel.**

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2000 **Chapter 4**

2001 **The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast**
2002 **Atlantic around Scotland.**

2003

2004 **Abstract**

2005 Microplastics have been widely documented to be accumulating in the marine environment. It is
2006 therefore of great importance to assess the uptake of microplastics in marine biota. This is the first
2007 study to report microplastic uptake in fish found in Scottish marine waters. Two sampling periods
2008 were investigated; the first in 2013 consisted of three demersal flatfish species (n = 128) collected
2009 from the east and west coasts of Scotland. The second collected in 2014 consisted of 5 pelagic
2010 species and 4 demersal species (n = 84) collected from the Northeast Atlantic. From the first
2011 sampling period, 48.4% of the gastrointestinal tracts of the demersal flatfish contained plastic of
2012 some size, with 39.1% having ingested microplastic. The average number of plastic items found
2013 per fish was 1.9 (\pm 0.9) with polyamide (65.5%), polyethylene terephthalate (14.7%) and acrylic
2014 (14.7%) being the three most commonly found plastics. There was no significant difference in
2015 plastic ingestion between the 3 demersal species from the first sampling period. From the second
2016 sampling period of the 84 pelagic and demersal fish caught, only 2 (2.4%) individuals from
2017 different species had ingested plastic. These two items were identified as a clear polystyrene fibre
2018 and a black polyamide fibre. Although macroplastic & microplastic uptake can be high in marine
2019 fish species this can vary across species and habitat with more work needing to be undertaken to
2020 determine the species and habitats at greatest risk.

2021

2022 **Keywords:** Macroplastic, Microplastic, Fish, Ingestion, Northeast Atlantic

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This chapter is a reformatted copy of my manuscript submitted 17/02/2017 to Marine Pollution Bulletin: **Murphy, F., Russell, M., Ewins, C., Quinn, B. The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland.** I was lead author on this paper and carried out all sample analysis and identification. Russel, M., provided sample catch data. Ewins, C. aided in the identification of samples. All authors provided comments and edits to create the final manuscript.

4.1. Introduction

Plastic has become a vital part of modern life and has grown in production from 1.7 million tonnes in 1950 to an estimated 322 million tonnes worldwide annually in 2015 (PlasticsEurope, 2016). Plastics represent a wide range of synthetic material that is cheap, persistent and lightweight (Derraik, 2002). It is for these reasons, amongst others that plastic pollution has become a major threat to the marine environment (Wilber, 1987, Derraik, 2002). Due to its light weight nature it can travel far from its original source covering vast distances being carried by wind and ocean currents and its durability means it can take many years to fully breakdown (Singh and Sharma, 2008). The impact of plastic on marine mammals (Laist, 1987, Laist, 1997), turtles (Mrosovsky et al., 2009) and seabirds (Azzarello and Van Vleet, 1987) has been widely documented for a number of years.

Attention has turned to the threat of much smaller pieces of plastic known as microplastics (Thompson et al., 2004). Microplastics are any piece of plastic < 5 mm in size (Arthur, 2009) and can be separated into two different types, primary microplastics and secondary microplastics. Primary microplastics are plastics that are designed to be of a microscopic size. Primary microplastics include pre-production pellets or nurdles used in the plastic manufacturing industry as well as microbeads used in personal care products as an abrasive material (Costa et al., 2010, Napper et al., 2015). Secondary microplastics are formed through the degradation of larger plastic material by environmental stressors such as sunlight, wind, rain and wave action (Singh and Sharma, 2008). Most microplastics in the marine environment originate from land based sources that are transported off shore (Jambeck et al., 2015). Waste water treatment works have also been shown to release microplastics into the environment in treated effluent (Browne et al., 2011, Murphy et al., 2016). Discarded fishing nets and line made of plastic are also a source of microplastics in the environment (Andrady, 2011).

Many marine organisms with differing feeding behaviours are known to ingest microplastics (Boerger et al., 2010, Murray and Cowie, 2011, Lusher et al., 2013) . Due to their size, microplastics may be more bio available to lower trophic organisms, which tend to display limited food selectivity and will ingest any item of appropriate size (Cole et al., 2013, Moore, 2008). There has been a number of studies looking at the uptake in fish (Lusher et al., 2013, Neves et al., 2015, Romeo et al., 2015, Bellas et al., 2016, Lusher et al., 2016, Nadal et al., 2016) showing

that a wide range of species from various geographical locations and depths are interacting with microplastic in the environment. The rate of uptake differs with species and location for example 17.5% of demersal fish (n = 212) consisting of 3 different species sampled from the Spanish Atlantic and Mediterranean coasts had ingested microplastic (Bellas et al., 2016). While 35% of demersal fish (n = 279) sampled from the English Channel consisting of 5 species had ingested plastic over 90% of which was < 5mm (Lusher et al., 2013), with ingestion rates ranging from 23.5 to 51.5%. There is also the issue of differing techniques used to extract and identify microplastic from fish tissue, for example relying solely on visual identification has the potential to overestimate the amount of microplastic present (Hidalgo-Ruz et al., 2012, Rocha-Santos and Duarte, 2015) while digestion methods have the potential to destroy microplastics that are present.

The impact of microplastics on fish is not fully understood, but negative effects have been observed in fish larva exposed to environmentally relevant concentrations of polystyrene microspheres (Lönnstedt and Eklöv, 2016). European perch (*Perca fluviatilis*) larva exposed to environmentally relevant concentrations of microplastic were found to exhibit lower hatching rates, lower activity rates and lower survival rates compared to the control (Lönnstedt and Eklöv, 2016). Trophic level transfer has been observed in crabs that have been fed mussels exposed to 0.5 µm fluorescent polystyrene microspheres (Farrell and Nelson, 2013), these microspheres were capable of being transported into the haemolymph and tissue of the crab. Exposure to microplastics has also been found to reduce deposit feeding marine worms energy reserves by up to 50% (Wright et al., 2013). The collection of environmentally relevant data is important as this will help guide toxicology testing in determining the actual effects of microplastics in a way that reflects what is happening in the environment (Rochman, 2016). It is therefore vital to determine the extent that marine organisms are ingesting microplastics. The occurrence of macroplastic & microplastic has been observed to be widespread in the sub-surface waters of the Northeast Atlantic (Lusher et al., 2014), with microplastic concentrations of 2.46 ± 2.43 per m³ calculated. The uptake of microplastics has previously been investigated in *Nephrops norvegicus* located in the west coast of Scotland, where high rates of uptake (83%) were observed (Murray and Cowie, 2011) as well as differences in uptake based on location (Welden and Cowie, 2016a). However, no studies have been carried out on fish species in the Northeast Atlantic around Scotland despite these high rates of microplastic uptake in *Nephrops norvegicus* and this being an important fishing ground.

In this study we investigate the presence of microplastics in demersal and pelagic fish taken from around the Scottish waters of the Northeast Atlantic. The aims of this study were: (i) to determine if fish present in Scottish waters are ingesting macroplastic and microplastics, (ii) to identify the types of polymers that are found, (iii) to determine differences in macroplastic and microplastic uptake in different species and (iv) to attempt to identify the potential sources of these macroplastic and microplastics.

4.2. Materials & Methods

4.2.1 Sample Collection

Fish samples were collected during two separate time periods, see Table 4.1 for latitude & longitude data for sampling sites. Fish sampled in 2013 in Scottish coastal waters consisted entirely of demersal species while the 2014 samples collected further offshore were a mixture of pelagic and demersal species (Figure 4.1). Fish had their entire gastrointestinal tracts dissected on the vessel, individually placed in plastic bags and immediately frozen at -20°C until analysis.

4.2.1.1 2013 Sampling

Fish were collected using a bottom trawl from the coastal waters near the east (Firth of Forth) and west (Clyde Estuary and Firth of Clyde) of Scotland (Figure 4.1) by Marine Scotland Science (MSS) between November and December 2013. Three species of flatfish were collected at depths between 8 to 78 m (Table 4.2).

4.2.1.2 2014 Sampling

Samples were collected from various locations in the Northeast Atlantic Ocean in September 2014 via bottom trawl, consisting of five pelagic species and four demersal species at depths between 300 to 1010 m (Table 4.2). The second set of samples consisted of a much greater variety of species but with fewer individuals per species than the first set.

4.2.2 Sample Processing

In the laboratory, samples were defrosted over ice and using clean scissors and forceps the gastrointestinal tracts were dissected and examined under a dissection microscope (Lusher et al.,

2112 2013). After examining the tissue thoroughly, the contents were washed with doubly distilled H₂O
2113 and re-examined to dislodge and clean any potential macroplastic or microplastic that had been
2114 obscured by the gastrointestinal tissue or contents. This was undertaken in a clean laboratory
2115 following a strict contamination protocol (Murphy et al., 2016). Briefly, all equipment used was
2116 cleaned and examined under a dissection microscope, clean cotton lab coats were worn at all times
2117 and all work surfaces were cleaned thoroughly before use. Any non-prey item, which is any item
2118 that did not appear to be part of the natural diet of the sample or appeared to be synthetic in nature,
2119 was removed and placed on a clean filter paper and sealed in plastic petri dish for further
2120 examination by micro Fourier Transform Infrared (FTIR) spectrometry.

2121 **4.2.3 Identification: Fourier Transform Infrared (FTIR) spectrometry**

2122 All potential macroplastic and microplastics found were examined under a dissection
2123 microscope, described by their morphology (fibre, bead, flake, etc) and colour, photographed and
2124 then positively identified using micro FTIR. A Perkin Elmer Spectrum One FTIR Microscope
2125 (manufactured in Llantrisant, United Kingdom) was used in the reflection mode using gold-coated
2126 glass microscope slides. Infrared radiation from 400 – 4000 cm⁻¹ was used allowing for the
2127 identification of chemical bonds present in the samples and also giving a characteristic signal in
2128 the “fingerprint” region. Using this technique and with the aid of a library of reference spectra
2129 polymers could be identified (Murphy et al., 2016). Samples of the plastic bags used to store the
2130 gastrointestinal tracts were analysed in order to exclude them as a potential source of
2131 contamination.

2132 **4.2.4 Statistics**

2133 Statistical analysis was conducted using R Studio version 3.2.2 statistical computing
2134 software. Data were tested for normality and homogeneity of variance. Differences in the number
2135 of fish to have ingested plastic and the number of items found between species were determined
2136 using one-way ANOVA's. A Pearson moment correlation was conducted on the gastrointestinal
2137 weight and the number of plastic items found in the fish containing macroplastic and microplastic.

4.3. Results

4.3.1 2013 Sampling

From the 128 demersal flat fish, 62 (48.4%) had ingested plastic of some size. In total 116 macroplastic and microplastic items were identified ranging in size between 0.1 mm – 15 mm (Table 4.3), 87 of which were below 5 mm in size and were found in 50 (39.1%) of the fish samples. Polyamide was the most common polymer found (Table 4.3) followed by polyethylene terephthalate (PET), acrylic, polypropylene (PP), and one item was found to be a mixture of PET and PP (Figure 4.2). Polyamide was found in all sites while acrylic was only found in plaice samples from Garroch Head, Holy Loch and Hunterson (Figure 4.3). There was no significant difference between plaice and flounder ($p = 0.63$), flounder and dab ($p = 0.85$) or plaice and dab ($p = 0.98$) in the uptake of macroplastic and microplastic (Figure 4.4). Fibres were the most commonly found type of microplastic (75.9%) followed by beads (18.4%) and flakes (4.6%) while one tubular item was found. Black (42.9%) was the colour most commonly found followed by clear (21.4%), blue (13.4%), green (11.6%), red (9.8%) and white (0.9%) (Figure 4.5). Of the flounder and plaice that contained plastic, the mean number of items found was 1.9 (± 0.9). There was no correlation between the number of macroplastic and microplastic items present and the gastrointestinal weight ($p = 0.96$).

4.3.2 2014 Sampling

From the 84 pelagic and demersal fish gastrointestinal tracts sampled only 2 (2.4%) individuals, a greater argentine and a megrim had ingested plastic (Figure 4.4). Two items were identified as plastic, a clear polystyrene fibre and a black polyamide fibre both > 5 mm in size.

4.4. Discussion

This is the first study to show the uptake of microplastics by fish in Scottish waters, with four demersal and one pelagic species found to have ingested plastic of some size. The results show that a range of fish species located in several locations in Scottish waters are ingesting macroplastic & microplastic. Ingestion rates of microplastic are as high as 39.1% in some species, rising to 48.4% when all plastic ingested is included. There was a clear difference in the uptake

rate of plastics between the two sampling periods. The 2013 sampling was conducted in shallow coastal waters (8 to 78 m depth) in areas with high anthropogenic activity. Sampling sites are located off the coast of what is known as the central belt of Scotland, an area of Scotland containing 3.5 million people (70% of the population) in an area of 10,000 m². The high level of urbanisation may result in high rates of plastic pollution due to debris being transported off shore or entering through wastewater effluent (Murphy et al., 2016). For example, Murphy et al., (2016) determined the amount of microplastic being released from a wastewater treatment works into the River Clyde finding that polyamide and acrylic combined made up 32% of the microplastic being released. Polyamide and acrylic were the two most commonly found microplastics in the west coast samples in the current study and is also the area where the River Clyde meets the sea. This area of the Scottish coast is relatively sheltered particularly the sites on the west coast compared to the 2014 sampling location. On the west coast of Scotland, there are high levels of marine recreational activity as well as a substantial aquaculture and marine fishing presence. However, although these are all potential sources of microplastic pollution it is difficult to determine the exact source of the polymers identified.

The 2014 samples were located further off shore in the Northeast Atlantic Ocean in much deeper water (209 to 1010 m) (Figure 4.1). Low microplastic uptake rates have previously been observed in demersal and pelagic fish sampled from the North Sea and the Baltic Sea with just 16 (5.5%) of the 290 fish sampled having ingested macroplastic or microplastic (Rummel et al., 2015). This is quite similar to the results found in the current study where just 2.4% of fish sampled in 2014 contained macroplastic or microplastic. The 2014 sampling sites are located far from high levels of urbanisation, which may result in significant dispersal of the macroplastic and microplastics originating from on shore activities and help to explain the relatively low concentrations found at these sites. Differences in the uptake of macroplastic and microplastic could also be due to the different species that were caught from these two distinct sampling sites. The 2013 samples consisted entirely of bottom feeders inhabiting areas with considerable anthropogenic inputs. Microplastic originating from anthropogenic activity may settle and accumulate in the sediment where these species reside resulting in the higher amounts of ingestion. The areas near the Shetland Islands and North of Scotland are much less populated and rural than the areas of central western and eastern Scotland.

Microplastic sub-surface water concentrations have been observed to be higher in coastal areas of the Northeast Pacific, with concentrations decreasing further offshore (Desforges et al., 2014). However, Lusher et al., (2014) recorded significantly higher sub-surface concentrations of microplastic in off shore Northeast Atlantic locations compared to coastal locations. Although sub-surface waters off the coast of Scotland were sampled by Lusher et. al., (2014), they were mainly confined to the Northwest of Scotland with no sampling near the Firth of Clyde undertaken. Difference in the uptake of plastic in fish found in urban rivers compared to rivers in areas with low anthropogenic activity has been observed previously in gudgeons (Sanchez et al., 2014).

A study conducted in the English Channel also looked at microplastics in demersal and pelagic fish finding that 35% of demersal samples had ingested plastic (Lusher et al., 2013). This is 18% lower than in the 2013 samples in this study, however of the five demersal species sampled in the English Channel one species (*A. cuculus*) was found to have an ingestion rate as high as 51.5%. Pelagic fish were also sampled from the English Channel and 38% were found to contain plastic. This is much higher in the current study, where only a single pelagic fish was found to have ingested plastic. This may be due to the much smaller number of individuals collected from each trawl in the 2014 samples where for most species only 10 individuals were collected. The average number of plastic pieces found in each demersal fish (1.90 ± 0.10) was similar to the 2013 samples (1.90 ± 0.9). Lusher et al. (2013) also found fibres to be the most common type of plastic found (68.3%) which was lower than the current study (75.9%). The ingestion rate in the present study was also higher than that found in mesopelagic and epipelagic fish sampled from the North Pacific Central Gyre (35%) (Boerger et al., 2010), however the mean number of plastic items per fish was similar (2.1 ± 5.78).

The only other studies examining plastic uptake in Scottish waters found that 83% *Nephrops norvegicus* (*Nephrops*) sampled in the Firth of Clyde had ingested plastic (Murray and Cowie, 2011) and that there were differences in the microplastic uptake in *Nephrops* based on location (Welden and Cowie, 2016a), with uptake rates ranging from 29% to 84%. The higher rates of microplastic uptake were observed in an area in close proximity to microplastic sources and human activity (Welden and Cowie, 2016a). These uptake rates are considerably higher than those found in the species examined in the current study. This may be due to differences in feeding behaviour, which may make *Nephrops* much more prone to the uptake of microplastics or

differences in retention time. High uptake rates of microplastic were also observed in another crustacean species, brown shrimp (*Crangon crangon*) sampled from coastal waters of the Southern North Sea and the English Channel (Devriese et al., 2015) where 63% of individuals sampled contained microplastic, consisting almost entirely of fibres (96.5%).

The effects of the ingestion of microplastic on marine biota is not well understood. A study looking at the effects of microplastics on the health and mortality of *Nephrops* (Welden and Cowie, 2016b), showed mortality increased in *Nephrops* fed 1.5 g of squid mantle spiked with 5 polypropylene fibres (41.6%) compared to the control fed 1.5 g squid mantle only (33.2%). Plastic exposed groups also exhibited reduced growth compared to the control, as well as lower feeding rates and reduced nutrient uptake.

Recent work has shown that environmentally relevant concentrations of microplastics can reduce hatching, activity and survival rates in fish larva (Lönnerstedt and Eklöv, 2016). This has the potential to interfere with the health and sustainability of fisheries exposed to microplastic. A study conducted on polychaete worms (*Arenicola marina*) chronically exposed to 5% unplasticised polyvinylchloride (UPVC) by weight significantly reduced feeding compared to the control and worms exposed to only 1% UPVC (Wright et al., 2013). Available energy reserves were reduced in worms exposed to 1% and 5% UPVC, by up to 50%. Exposures to microplastic also led to an increase in the inflammatory response in the worms exposed to 5% UPVC, a metabolically demanding process. The time between ingestion and egestion events took 1.5 times longer in worms exposed to microplastic. A pelagic copepod (*Calanus helgolandicus*) exposed to 20 µm polystyrene beads for 24 hrs was found to ingest 11% fewer algae cells and sustained exposure resulted in significant reduction in reproductive output (Cole et al., 2015). This indicates that ingested microplastic has the potential to affect the health of organisms through reducing feeding or increasing metabolic demand. However, it is not fully understood whether this is applicable to fish that have ingested microplastic or if the concentrations present in the environment are capable of causing these negative effects. The potential impact of microplastic ingestion on commercial fisheries in Scotland is not well studied but may effect fitness which could have resultant effects on fecundity and the sustainability of populations particularly in Scottish coastal waters (Welden and Cowie, 2016a).

The physical blockage of macroplastic and microplastic is also a concern, ingested plastic may become lodged in the digestive tract preventing subsequent ingestion and egestion. Figure 4.2 shows a tangled ball of fibres which was isolated in a single fish from the 2013 samples, completely blocking the gastrointestinal tract. There is also the potential for ingested microplastic to give a false sense of satiation and cause a reduction in feeding. The uptake of microplastic may also increase metabolic demand due to the need to ingest/egest the microplastic or the time to process the microplastic as it travels through the digestive system and is eventually excreted.

The effect of the physical uptake of microplastic by fish is not the only concern, the uptake of harmful contaminants potentially absorbed on to the surface of the microplastics is a major issue (Velzeboer et al., 2014). Polyethylene beads were allowed to absorb environmental pollutants, these beads were then used in exposures on fish (*Oryzias latipes*) (Rochman et al., 2013). Exposed fish, were found to be able to bio-accumulate these chemicals and this caused liver toxicity and pathology. Microplastic co-contaminants have the potential to affect exposed fisheries health and sustainability representing another pressure to already threatened fish stocks (Hutchings and Reynolds, 2004). The uptake of microplastics and sorbed co-contaminants by commercially caught fish represents a risk to human health as these contaminants may transfer to fish tissue and eventually humans through consumption (Galloway, 2015). In future studies, it may be important to not only identify the type of macroplastic and microplastic ingested but to attempt to measure chemicals that may have sorbed on to the surface of the macroplastic and microplastic to fully understand the potential risk facing marine biota.

4.5. Conclusions

This study adds to the existing evidence that macroplastic and microplastic is taken up by a range of fish species from various locations. Fish in Scottish marine waters are ingesting macroplastic and microplastic with a variety of polymers identified. Ingestion was much higher in species found in shallower coastal waters than species in deeper further offshore waters. The variability in ingestion rates across geographical regions and species presents difficulties in determining the risk that marine biota face from macroplastic and microplastic pollution. This variability could be due to differences in feeding behaviour, preferred habitat and the effects of wind and ocean currents transporting plastic debris. All these factors may contribute to the uptake

of macroplastic and microplastics by marine biota, it is therefore important that as wide a range of species and habitats are investigated for the uptake and presence of macroplastic and microplastics.

Acknowledgements

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2314 **Table 4.1. Latitude & longitude data for 2013 & 2014 sampling sites (B = Bowling; HL =**
 2315 **Holy Loch; GH = Garroch Head; H = Hunterson; SAB = St. Andrews Bay; OF = Outer**
 2316 **Forth)**

Year	Latitude	Longitude	ID
2013	55.9285	-4.4845	B
2013	55.9539	-4.9036	HL
2013	55.6828	-5.0342	GH
2013	55.7488	-4.8962	H
2013	56.3536	-2.7493	SAB
2013	56.1180	-2.5349	OF
2014	61.6073	-1.0568	355
2014	61.6462	-1.7127	356
2014	61.1980	-2.7010	363
2014	60.9328	-2.3938	366
2014	59.8767	-5.2342	370
2014	60.1465	-5.1818	372
2014	59.1405	-9.8707	378
2014	59.3735	-10.1197	380

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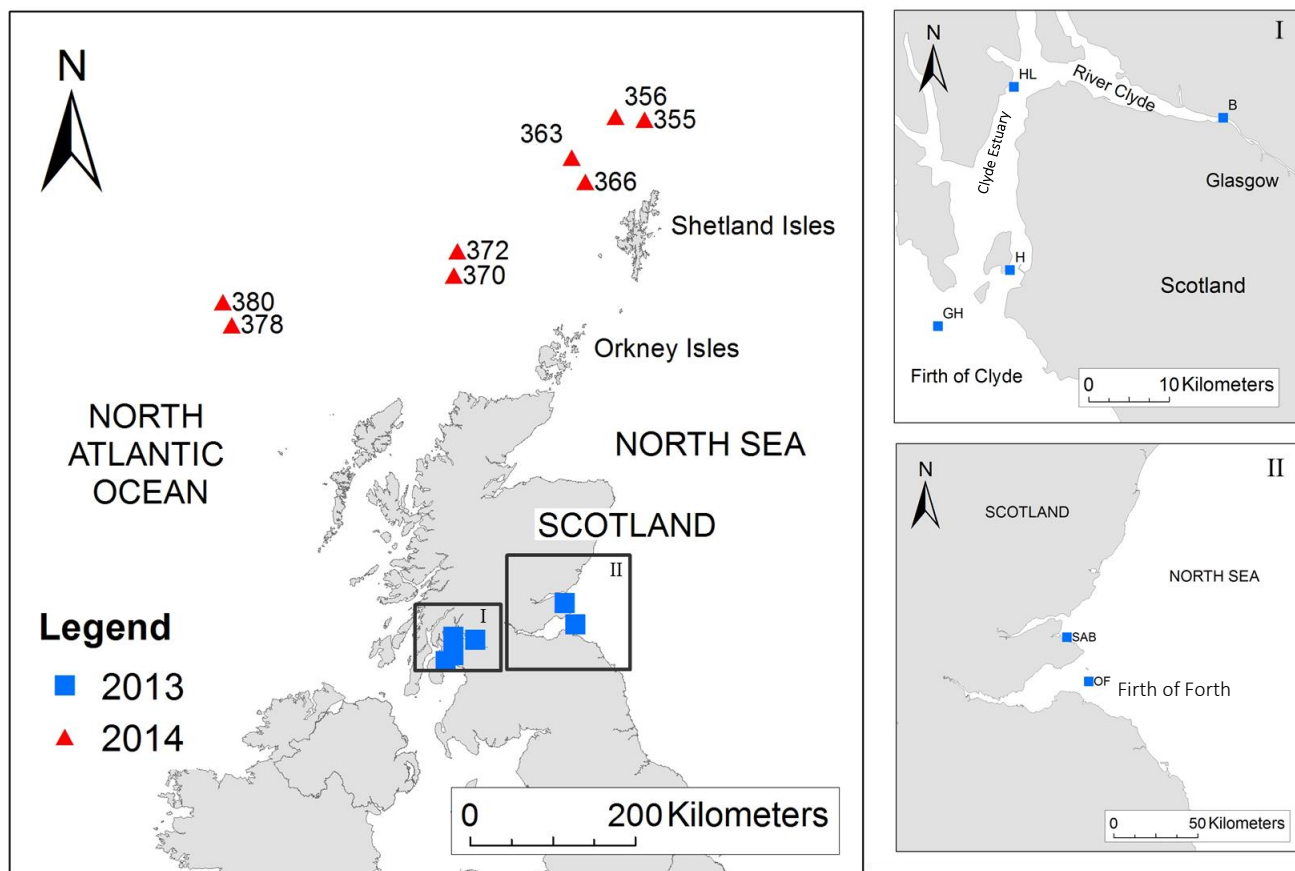


Figure 4.2. Map of sampling sites based on GPS data taken during the 2013 & 2014 Marine Scotland Science sampling (B = Bowling; HL = Holy Loch; GH = Garroch Head; H = Hunterson; SAB = St. Andrews Bay; OF = Outer Forth)

Table 4.2. List of species sampled in 2013 & 2014 trawls and the number (n) of each species sampled.

Year	Species	Common name	n	Habitat
2013	<i>Pleuronectes platessa</i>	Plaice	62	Demersal
	<i>Platichthys flesus</i>	Flounder	47	Demersal
	<i>Limanda limanda</i>	Common Dab	19	Demersal
2014	<i>Pollachius pollachius</i>	Pollock	5	Demersal
	<i>Molva molva</i>	Ling	5	Demersal
	<i>Hippoglossus hippoglossus</i>	Halibut	14	Demersal
	<i>Lepidorhombus whiffiagonis</i>	Megrim	10	Demersal
	<i>Micromesistius poutassou</i>	Blue Whiting	20	Pelagic
	<i>Argentina silus</i>	Greater Argentine	15	Pelagic
	<i>Trachurus trachurus</i>	Horse Mackerel	5	Pelagic
	<i>Aphanopus carbo</i>	Black Scabbard	5	Pelagic
	<i>Coryphaenoides rupestris</i>	Round Nose Grenadier	5	Pelagic

Table 4.3. Polymers found in 2013 demersal fish with all plastics (macroplastic & microplastic combined) and microplastics only shown (PET = polyethylene terephthalate, PP = polypropylene, Mix = Mixture of PET & PP).

2013	All Plastics		Microplastics	
Polymer	No	%	No	%
Polyamide	76	65.5	58	66.7
PET	17	14.7	16	18.4
Acrylic	17	14.7	10	11.5
PP	5	4.3	2	2.3
Mix	1	0.9	1	1.1
Total	116	100.0	87	100.0

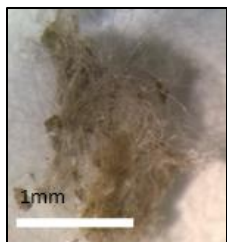


Figure 4.3. Photo of a ball of polyethylene terephthalate and polypropylene fibres from the gastrointestinal tract of a flounder taken during the 2013 sampling at the Outer Forth (OF).

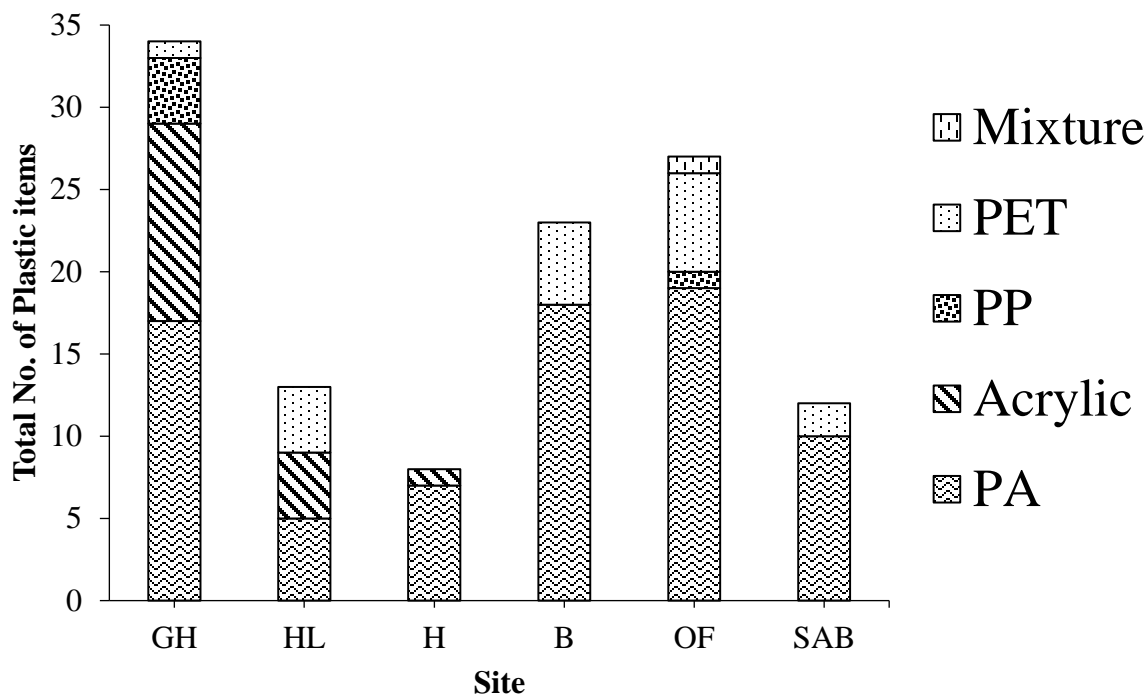


Figure 4.3. Number of macroplastic & microplastic items and their polymer type found at each site for the 2013 samples. GH = Garroch Head; HL = Holy Loch; H = Hunterson; B = Bowling; OF = Outer Forth; SAB = St Andrews Bay. The polymer types are: PET = polyethylene terephthalate, PP = polypropylene and PA = polyamide.

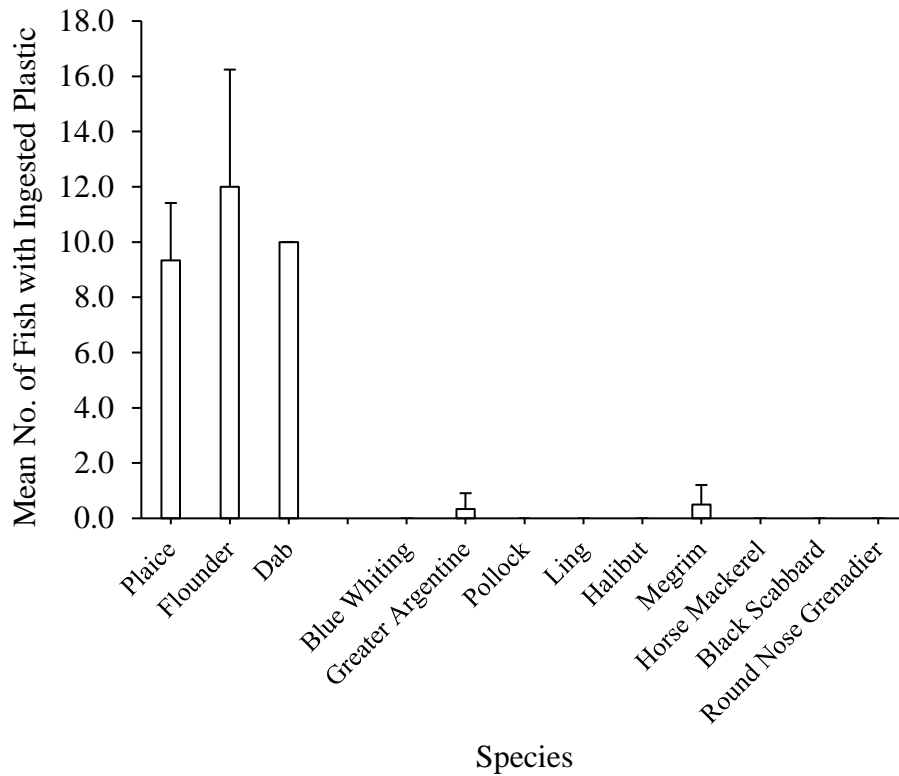


Figure 4.4. Barchart of mean number of fish with ingested plastic (macroplastic & microplastic) per species. Error bars = standard deviation (Note Dab (*L. limanda*) has no error bars as only one site contained this species).

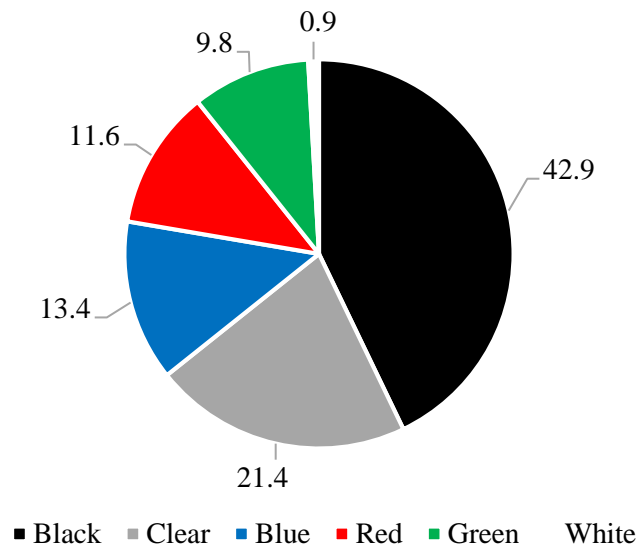


Figure 4.5. Pie chart showing the abundance of each colour of plastic (macroplastic & microplastic) found from the 2013 samples as a percentage.

Chapter 5

The effects of microplastic on freshwater *Hydra attenuata* feeding, morphology & reproduction.

Abstract

Microplastic pollution has been a growing concern in the aquatic environment for several years. The abundance of microplastics in the environment has invariably lead them to come into contact with a variety of different aquatic species, many of whom can ingest these contaminants the impact of which is not fully understood. Much of the research on microplastic pollution has focused on the marine environment and species with little research undertaken in freshwater. Here we examine the effect of microplastics on the freshwater cnidarian, *Hydra attenuata*, this study also describes the development and use of a bioassay to investigate the impact of microplastic on freshwater organisms. *H. attenuata* play a vital role in the planktonic make up of slow moving freshwater bodies which they inhabit and are sensitive environmental indicators. *H. attenuata* were exposed to polyethylene flakes ($< 400 \mu\text{m}$) extracted from facewash at different concentrations (Control, 0.01, 0.02, 0.04, 0.08 g mL^{-1}). The ecologically relevant endpoint of prey (*Artemia salina*) and microplastics ingestion rates were recorded at 30 min and 60 min. After which *H. attenuata* were transferred to clean media and observed after 3, 24, 48 & 96 hrs with changes in their morphology and reproduction (Hydranth numbers) recorded. The results of this study show that *H. attenuata* are capable of ingesting microplastics, with several individuals completely filling their gastric cavities. Significant reductions in feeding rates were observed after 30 min in 0.02 & 0.08 g mL^{-1} and after 60 min in 0.04 & 0.08 g mL^{-1} exposures. Exposure to the microplastics caused significant changes to the morphology of *H. attenuata*, however these changes were non-lethal. This study demonstrates that freshwater *H. attenuata* is capable of ingesting microplastics and that microplastic can significantly impact the feeding of freshwater organisms.

Keywords: Microplastic, *Hydra attenuata*, Feeding, Microbead, Polyethylene

This chapter is a reformatted copy of my manuscript submitted 31/01/2017 to Environmental Pollution: Murphy, F. & Quinn, B. The effects of microplastic on freshwater *Hydra attenuata* feeding, morphology & reproduction. I was lead author on this paper and carried out all exposures. I was lead author and designed and carried out all exposures. Quinn, B. aided in the experimental design and provided comments and edits to help create the final manuscript.

5.1. Introduction

Plastic pollution in the environment has been well studied for a number of decades (Azzarello and Van Vleet, 1987, Pruter, 1987, Derraik, 2002). The impact of larger plastic material on birds (Azzarello and Van Vleet, 1987), marine mammals (Laist, 1997) and turtles (Tomás et al., 2002) has been given considerable attention. In recent years the issue of smaller plastic material known as microplastics has been gaining increasing attention (Andrady, 2011). Microplastics are pieces of plastic < 5 mm (Arthur, 2009) and have been found in sediments (Browne et al., 2011, Eriksen et al., 2013a), aquatic water bodies (Collignon et al., 2012, Lechner et al., 2014, Free et al., 2014) and ingested by a range of species with varying feeding strategies and habitats (Lusher et al., 2016, Welden and Cowie, 2016a). The study of microplastic pollution has primarily focused on the marine environment with comparatively little research conducted on the freshwater environment, however research is showing that microplastic pollution of the freshwater environment may be as prevalent, as reviewed by (Eerkes-Medrano et al., 2015).

Sources of microplastic in the freshwater environment include treated effluent from wastewater treatment plants (WWTP), with one plant in Scotland estimated to release up to 65 million microplastics into the freshwater/brackish environment everyday (Murphy et al., 2016). A number of lakes have been investigated for microplastic pollution (Eriksen et al., 2013a, Imhof et al., 2013, Free et al., 2014). The Great Lakes in North America for example, were found to have an average concentration of 43,157 particles per km⁻² with the most populated lake found to have the highest microplastic count (Eriksen et al., 2013a). Research undertaken on microplastic ingestion by freshwater organisms in natural populations (Faure et al., 2012, Sanchez et al., 2014, Biginagwa et al., 2016) found 12% of wild gudgeons sampled from French rivers (Sanchez et al., 2014) and 20% of Nile perch and Nile *tilapia* purchased in a harbour market in Lake Victoria contained microplastic (Biginagwa et al., 2016).

Several studies have looked at the potential uptake and effects of microplastics on freshwater organisms in the laboratory, these include invertebrate and vertebrate species (Rosenkranz et al., 2009, Imhof et al., 2013). Imhof et al., (2013) exposed a range of freshwater invertebrate species to microplastic and found 5 freshwater species capable of ingesting microplastic. *Daphnia* exposed to 20 nm and 1000 nm fluorescent polystyrene microspheres were found to uptake the spheres at concentrations of 2 µm L⁻¹ (Rosenkranz et al., 2009). When placed in clean water after 4 hrs of exposure 90% of the 1000 nm microspheres were cleared from the *Daphnia* and only 40% of the 20 nm in the same period. Despite its

prevalence in the environment and the growing concern over its potential harmful effects there is currently no standardised bioassay for determining the toxicity of microplastic.

In the present study, we describe the development and use of a bioassay to investigate the impact of microplastic on the freshwater cnidarian *Hydra attenuata*. *H. attenuata* inhabits slow moving freshwater bodies where they regulate the planktonic structure of these habitats (Burnett, 1973). *H. attenuata* reproduce asexually by budding and reproduce every three days provided there is an adequate food supply (Burnett, 1973). *H. attenuata* is easily cultured and maintained in the laboratory and has been used extensively in toxicological assays as they are sensitive environmental indicators (Quinn et al., 2008a). The effects of waste water, pharmaceuticals, and heavy metals on *H. attenuata* have all been investigated (Karntanut and Pascoe, 2002, Quinn et al., 2004, Quinn et al., 2008a). A modified version of a previously developed protocol (Quinn et al., 2008a), was used to determine the impact of microplastic exposure on the ecologically relevant endpoints of (i) feeding rates (ii) morphology (based on the Wilby, 1988 scoring system) and (iii) hydranth number (indicating reproduction)

5.2. Materials & Methods

5.2.1 Test Organism

Cultures of *H. attenuata* were sourced from a population in the Environment Canada St-Lawrence Centre (SLC), Montreal, Quebec, which have previously been used in various toxicity studies (Blaise & Kusui, 1997, Trottier et al., 1997, Quinn et al., 2007). *H. attenuata* were cultured in glass bowls containing 700 mL of Hydra medium (147 mg L⁻¹ CaCl₂2H₂O, 110 mg L⁻¹ 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl) amino] ethanesulfonic acid, pH 7) at 18 °C ± 2 °C with an 8 hr light and 16 hr dark photoperiod, following the procedure described by Trottier et al., (1997) and were fed freshly hatched *A. salina* daily. All *H. attenuata* selected for the exposures had a morphological score of 10 per Wilby (1988) scoring system. Briefly the scoring system determines toxicity by measuring drastic changes in morphology by observing the contraction of tentacles and the body and is scored from 10 (healthy, elongated tentacles and body) to 0 (disintegration). Scores of 10 – 6 (sub-lethal signs of toxicity such as shortened and clubbed tentacles) are reversible while scores of 5 and below are irreversible and considered endpoints of lethality.

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2514 **5.2.2 Microplastic**

2515 Polyethylene flakes were sourced from a commercially available face wash product. The face wash
2516 was passed through a 400 µm sieve to remove larger pieces of microplastic. A size class of < 400 µm was
2517 chosen as the freshly hatched *Artemia nauplii* that are fed to the *H. attenuata* are < 400 µm in size. The
2518 microplastics extracted were irregularly shaped, were blue and clear in colour and their polymer type was
2519 confirmed using Fourier Transform Infrared spectrometry (FTIR). The extracted microplastic were
2520 washed 3 times with 70% ethanol, distilled H₂O and Hydra media then dried before the amounts used
2521 were weighed. The concentrations used in all the exposures were Control, 0.01, 0.02, 0.04, 0.08 g mL⁻¹.

2522 **5.2.3 Exposures and Endpoints**

2523 Two separate exposures were carried out in 0.5 & 2 mL plastic microcentrifuge tubes (Fisher
2524 Scientific). The relevant concentration of microplastic was weighed and placed into each tube, that was
2525 then filled with Hydra media and inverted 10 times to ensure the microplastic was homogenously mixed.
2526 Healthy (morphology score 10) individual *H. attenuata* with 2 hydranths were selected from the
2527 population and carefully added to each tube (n=3 per tube) using a pipette with each concentration being
2528 undertaken in triplicate (n=9), the 0.5 mL exposure was duplicated (n=18). *A. salina* was washed three
2529 times in Hydra media and 10 healthy (swimming) individuals were added to each microcentrifuge tube,
2530 care was taken to avoid adding *A. salina* directly onto the *H. attenuata* tentacles. The exposures began
2531 when the microcentrifuge tubes were added to the apparatus used to mechanically mix the tubes.

2532 Two different methods of agitating the microcentrifuge tubes to keep the microplastic in
2533 suspension were tested, a shaker (Stuart Shaking Incubator SI500) at 75 rpm used for the 0.5 mL tubes
2534 and a mechanical rotator that was used to invert the 2.0 mL tubes. Microplastic ingestion and feeding rates
2535 were recorded after 30 min and 60 min, after which they were removed using a pipette, placed in a petri
2536 dish with clean Hydra media and observed under a dissection microscope. Microplastic ingestion was
2537 determined by counting the number of polyethylene flakes in the gastric cavity, while feeding rates were
2538 determined by counting the number of *A. salina* in the gastric cavity. For the recovery test, *H. attenuata*
2539 were transferred from the microcentrifuge tube and placed in a well of a 12 well multi-well plate with 2.0
2540 mL of media with three *H. attenuata* per well. Morphology score and hydranth number were recorded
2541 after 3, 24, 48 & 96 hrs.

5.2.4 Statistics

Statistical analysis was conducted using R studio version 3.2.2. All data was tested for normality using Shapiro Wilks test for normality and equal variance was tested using Bartlett's test. Differences in the number of microplastics ingested, feeding rates, morphology & hydranth number were determined using one way ANOVA. Feeding rates, morphological scores and hydranth number were all compared to the control to determine significance while microplastic ingestion was compared to the lowest concentration (0.01 g mL^{-1}) to determine significance. Pearson moment correlations were carried out on microplastic ingestion and feeding rates. IC_{50} values (that inhibits the feeding rate by 50%) were calculated using linear regression analysis.

5.3. Results

5.3.1 0.5 mL Microcentrifuge Tube Exposure

5.3.1.1 Microplastic Ingestion

There was a significant increase in the ingestion of microplastics in the highest concentrations compared to the lowest concentration after 30 min ($p = 0.002$) and 60 min ($p = 0.036$) in the 0.5 mL microcentrifuge tube exposures (Figure 5.1A). The mean number of microplastic particles ingested was significantly higher in the 0.04 g mL^{-1} concentration at the 30 min ($p = 0.015$, $n = 2.0 \pm 0.53$) time point and in the 0.08 g mL^{-1} concentration at both the 30 min ($p = 0.007$, $n = 2.2 \pm 0.75$) and 60 min ($p = 0.047$, $n = 2.6 \pm 0.85$) time points (Figure 5.1A).

5.3.1.2 Feeding Rates

There was a significant decrease in the feeding rates of *H. attenuata* exposed to microplastic after the 30 min ($p = 0.003$) and 60 min ($p = 0.002$) time points (Figure 5.1B). The mean number of *A. salina* ingested was significantly lower in the 0.02 g mL^{-1} concentration after 30 min ($p = 0.045$) and the 0.04 g mL^{-1} concentration after 60 min ($p = 0.045$), while the 0.08 g mL^{-1} concentration feeding rates were significantly lower at both the 30 min ($p = 0.002$) and 60 min ($p = 0.001$) time points. There was a significant negative correlation between the amount of *A. salina* ingested and the amount of microplastic

ingested at the 30 min ($p = 0.046$) and 60 min ($p = 0.003$) time points. The IC_{50} values calculated for the feeding rates were 0.0361 g mL^{-1} for the 30 min exposure and 0.0350 g mL^{-1} for the 60 min exposure.

5.3.1.3 Hydra Morphology & Hydranth Numbers

The morphology score of *H. attenuata* was significantly lower in the 30 min 0.08 g mL^{-1} exposure after 3 hrs ($p = 0.026$), with no other significant differences were observed in the 30 min exposure (Figure 5.2A). Morphological scores were significantly lower in the 60 min 0.08 g mL^{-1} exposure after 3 hrs ($p = 0.001$), 24 hrs ($p = 0.001$) and 48 hrs ($p = 0.022$) (Figure 5.2B). Hydranth numbers were only significantly lower in the 60 min 0.08 g mL^{-1} exposure after 96 hrs ($p = 0.051$) (Figure 5.2D).

5.3.2 2.0 mL Microcentrifuge Tube Exposure

5.3.2.1 Microplastic Ingestion & Feeding Rates

There was no significant difference in the ingestion of microplastic in either the 30 or 60 min exposures ($p > 0.05$) (Figure 5.3A). Feeding rates were significantly lower in the 30 min 0.04 g mL^{-1} ($p = 0.037$) and 0.08 g mL^{-1} ($p = 0.045$) exposures compared to the control (Figure 5.3B). No significant differences were observed in the 60 min exposure feeding rates ($p > 0.05$) and no feeding was observed at the 0.04 and 0.08 g mL^{-1} microplastic concentrations. There was a significant negative correlation between the amount of *A. salina* ingested and the amount of microplastic ingested ($p = 0.001$) in the 30 min exposure, there was no significant correlation in the 60 min exposure ($p = 0.183$).

5.3.2.2 Hydra Morphology & Hydranth Numbers

The morphological score in the 30 min exposure was only significantly lower in 0.08 g mL^{-1} after 24 ($p = 0.028$) and 48 ($p = 0.012$) hrs compared to the control (Figure 5.4A). The morphological score in the 60 min exposure was significantly lower in the 0.08 g mL^{-1} concentration at all time points ($p < 0.05$) (Figure 5.4B). Hydranth numbers were significantly lower in the 30 min 0.08 g mL^{-1} exposure after 48 ($p = 0.036$) and 96 ($p = 0.051$) hrs compared to the control (Figure 5.4C). While the hydranth number in the 60 min exposure was significantly lower in the 0.08 g mL^{-1} concentration after 24 ($p = 0.030$), 48 ($p = 0.026$) and 96 ($p = 0.050$) hrs (Figure 5.4D).

5.4. Discussion

As microplastics are ubiquitous in the environment and the amount generated is likely to increase as well as the increasing number of studies showing that aquatic biota is interacting with microplastics it is vital to be able to determine their toxicity. The development of a bioassay to assess the effects of microplastic is of great importance in determining what concentrations are of concern to the health of aquatic biota in the environment. *H. attenuata* is a freshwater organism used in standardised tests by organisations such as Environment Canada to test the toxicity of various pollutants (Blaise & Kusui, 1997, Karntanut and Pascoe, 2002, Quinn et al., 2008a). This was the primary reason that this species was chosen as the test organism in the present study. As we are attempting to develop a new technique, several methods of exposing *H. attenuata* to microplastics were investigated before the method used in the present study was finalised. To test the uptake of microplastic by *H. attenuata* an initial exposure involved placing *H. attenuata* in a petri dish with Hydra media spiked with commercially sourced (Cospheric®) florescent polyethylene microspheres. These polyethylene microspheres were within the size range of *H. attenuata* prey ($\leq 400 \mu\text{m}$) but had a uniform shape and size and were not ingested by the *H. attenuata*. The experiment was repeated using microplastics sourced from a commercially sold facewash product containing irregularly shaped polyethylene flakes which were thought to better resemble *H. attenuata*'s natural prey. This exposure showed that *H. attenuata* were capable of ingesting microplastic and these microplastic flakes were used in all subsequent exposures.

Preliminary exposures to microplastics and feeding tests were carried out in petri dishes and 12 well multi-well plates as per the previously published protocol (Trottier et al., 1997). However, as we were using polyethylene with a density lower than the Hydra media ($0.926 - 0.940 \text{ g cm}^{-3}$) the microplastics did not maintain a homogeneous mixture in suspension and by floating on the surface were physically removed from the test organism. To allow for a more homogenous mixture the exposure was carried out in 0.5 mL microcentrifuge tubes placed on a shaker which agitated the microplastic sufficiently to keep them in suspension and available to the *H. attenuata* resulting in mixing within the microcentrifuge tubes. A mechanical rotator which inverted the 0.5 mL tubes was also tested, but was deemed unsuccessful as little to no mixing of the microplastic was observed. Larger 2.0 mL microcentrifuge tubes were tested using the mechanical rotator and mixing was observed due to the presence of air bubbles. These two methods were then used in the final exposure studies. Over the course of the 2.0 mL exposure it became apparent that the mixing of the microplastic at the higher concentrations was causing physical damage to

the *H. attenuata* resulting in morphological impairment observed at these concentrations that invalidated the feeding test for these exposures.

In this study *H. attenuata* were observed to have significantly reduced feeding in both the 0.5 mL 30 & 60 min exposures (Figure 5.1B), with feeding rates significantly negatively correlated with microplastic ingestion. Exposure to microplastic has the potential to reduce the health of *H. attenuata* by impacting on its ability to feed and limiting the amount of prey consumed. This interaction could have a profound impact in the environment, not only on wild populations of *H. attenuata* but also on their prey species. Feeding is an important and ecologically relevant endpoint as fluctuations in feeding can have major effects on the fitness of individuals and reproduction as well as knock on effects to prey species populations (Kooijman, & Metz, 1984). These potential community level effects could have significant impacts on the stability of freshwater habitats.

It is somewhat difficult to compare these results with environmental data as this tends to be presented as microplastic counts rather than by weight. There is also the issue of different sampling methodology resulting in very different microplastic abundance estimates (Quinn et al., 2017). However, in order to test their impact relatively high concentrations of microplastic were used in this controlled bioassay compared to the quantities measured in most environmental samples. Sampling of the northeast Atlantic has shown there to be 2.46 ± 2.43 particles per m^{-3} (Lusher et al., 2014), which converts to 0.00000246 particles per mL^{-1} . It is unlikely that these particles numbers would weigh close to what was used in the current study considering the lowest concentration used (0.01 g mL^{-1}) would contain approximately 800 particles. However, organisms located close to sources of microplastic may experience significantly higher microplastic concentrations, for example a Swedish harbor located near a polyethylene production plant reported concentrations of 102,000 plastic particles per m^{-3} or 0.102 particles per mL^{-1} (Noren 2007). A predicted no effect concentration (PNEC) can be extrapolated by dividing the IC_{50} values by a factor of 1000 (Jones et al., 2002). If the measured environmental concentration (MEC)/PNEC value is <1 then no further assessment is necessary (Quinn et al., 2008b), the PNEC values for the 0.5 mL microcentrifuge tube exposure was calculated based on particle numbers at 30 and 60 min (Table 5.1). Using the environmental concentrations measured by Lusher et al., (2014) and Noren (2007), the MEC/PNEC values extrapolated produce values <1 indicating no further assessment is necessary (Table 5.1). Although still considerably lower than what was used in the current study, these MEC values demonstrate the great variability in microplastic concentrations in the environment. Both

MEC/PNEC particle number values calculated are well below 1 indicating that no further assessment is necessary (Table 5.1). However, due to the variability in microplastic morphology and polymer composition it is not possible to rule out the potential risk of other microplastics not investigated. Other environmental contaminants such as heavy metals (Brennecke et al., 2016) and persistent organic pollutants (Frias et al., 2010) also have the potential to absorb on to the surface of microplastics which may increase the risk to exposed organisms.

Although an impact on the *H. attenuata* morphology in the 0.5 mL microcentrifuge tubes was observed in the present study (Figure 5.2A & B), these changes were non-lethal and the *H. attenuata* would be able to recover. The effect of microplastic on freshwater invertebrate morphology has previously been looked at using mud snails exposed to concentrations of various polymers (Imhof and Laforsch, 2016). This study found almost no effect on adult morphology but did show some effect on juvenile development (Imhof and Laforsch, 2016). In the present study hydranth numbers (indicating reproduction) remained unchanged throughout apart from the 0.5 mL 60 min 0.08 g mL⁻¹ exposure after 96 hrs (Figure 5.3D), which was significantly lower than the control but did not fall below the number present at the beginning of the exposure.

H. attenuata was capable of readily ingesting microplastic (Figure 5.5), with some individuals completely filling their gastric cavity preventing ingestion of *A. salina*. The ingestion of microplastic may effect an organism in a number of ways, it may cause internal damage to the gastric cavity, a false sense of satiation and impairment of appendages (Gregory, 2009, Gall and Thompson, 2015). Normally it takes *H. attenuata* less than 8 hrs to expel any waste material from their gastric cavity, but in the current study this took considerably longer, up to 48h in some individuals to egest microplastic. These results indicate that when exposed to microplastics *H. attenuata* are expending considerably more time and energy clearing their gastric cavity then under normal conditions. During this time *H. attenuata* may not be able to feed normally as the gastric cavity is full potentially further impacting on their health. Microplastics were observed to stick to the tentacles of *H. attenuata* which could potentially impair feeding by restricting its ability to move and capture prey. The ingestion of high numbers of microplastic particles was also observed to cause *H. attenuata* to become positively buoyant making it increasingly difficult to remain attached to the substrate and liable to floating, again potentially impacting on its ability to feed.

As mentioned above, during the feeding tests the uniform microspheres were not ingested by *H. attenuata*, while the irregularly shaped facewash polyethylene flakes better resembling their prey (*A.*

salina) were readily ingested. This demonstrates the influence of the shape and size of the microplastics has on the uptake of microplastics in aquatic biota. The influence of microplastic size on uptake has been observed in *Daphnia* (Rosenkranz et al., 2009), *Daphnia* exposed to polystyrene beads were shown to uptake 1000 nm beads 40 times higher than 20 nm after 60 min. *H. attenuata* were also observed to ingest fibres in preliminary studies, this was the result of contamination occurring in the exposures and was not intentional. However, it is of importance as microplastic fibres can make up a considerable amount of the microplastic pollution entering the environment (Murphy et al., 2016, Napper & Thompson, 2016). The influence of microplastic morphology is an important factor that needs to be taken into consideration when designing microplastic exposure studies in order for a comprehensive assessment of the risks to be made.

The effects of microplastic ingestion has previously been investigated in the freshwater arthropod, *Gammarus fossarum* exposed to 2680 cm⁻² polyamide fibres for 0.5, 2, 8 and 32 hrs and 60,000 polystyrene beads mL⁻¹ for 24 hrs (Blarer & Burkhardt-Holm, 2016). *G. fossarum* were found to be capable of ingesting the polyamide fibres after 0.5 hrs, however half the individuals expelled the polyamide fibres after one hour in clean media. After 16 hrs, all polyamide fibres were expelled (Blarer & Burkhardt-Holm, 2016). The polystyrene beads were also ingested but the amounts were not counted only the presence or absence was reported (Blarer & Burkhardt-Holm, 2016). This demonstrates the difference that feeding mechanisms may have on the retention and speed of egestion/excretion of microplastic once ingested. The effects of plastic ingestion have been studied in adult *E. brasiliensis* fish, collected from the Goiana estuary (Ramos et al., 2012). Fish that had ingested plastic fragments were recorded as having lower mean total weights of gut contents. Previous studies on microplastic and freshwater invertebrates have primarily focused on the uptake of microplastic but not the effects (Rosenkranz et al., 2009, Imhof et al., 2013, Blarer & Burkhardt-Holm, 2016).

Studies investigating the effects of microplastic on freshwater organisms have been focused mainly on fish species (Ramos et al., 2012, Rochman et al., 2013, Oliveira et al., 2013). Laboratory studies have looked at the effects of microplastic over long time periods (Rochman et al., 2013). Japanese medaka exposed to both virgin and marine low density polyethylene (LDPE) over a two month period displayed signs of liver stress (Rochman et al., 2013). Severe glycogen depletion was observed in 74% of marine plastic exposed fish, 46% of virgin plastic fish and 0.5% of control fish. Fatty vacuolation was observed in 47% of marine plastic fish, 29% virgin plastic fish and 21% of control fish. Single cell necrosis was also observed in 11% of marine plastic fish and 0% of the virgin plastic and the control fish. Common

goby exposed to pyrene (20 and 200 μm) for 96hrs in the presence and absence of microplastics (0, 18.4 and 184 $\mu\text{g L}^{-1}$) (Oliveira et al., 2013). Microplastic combined with pyrene exposure decreased the energy available through the aerobic pathway of energy production. These studies demonstrate the risks of microplastic co-contaminants may have on freshwater organisms. Microplastics have the potential to act as sink of environmental contaminants resulting in them concentrating on to the surface of the microplastic (Bakir et al., 2012). These sorbed contaminants may subsequently be released from the microplastic once ingested resulting in toxic effects to the exposed organisms. The hydra bioassay developed in the present study could potentially be used to assess these microplastic co-contaminants in future studies.

5.5. Conclusions

This study adds to the growing body of research on the effects of microplastic on freshwater organisms. As freshwater habitats are already heavily stressed by anthropogenic activity (Strayer, 2006), it is of considerable importance that emerging contaminants such as microplastic are studied to determine their risk to freshwater biota.

Acknowledgments

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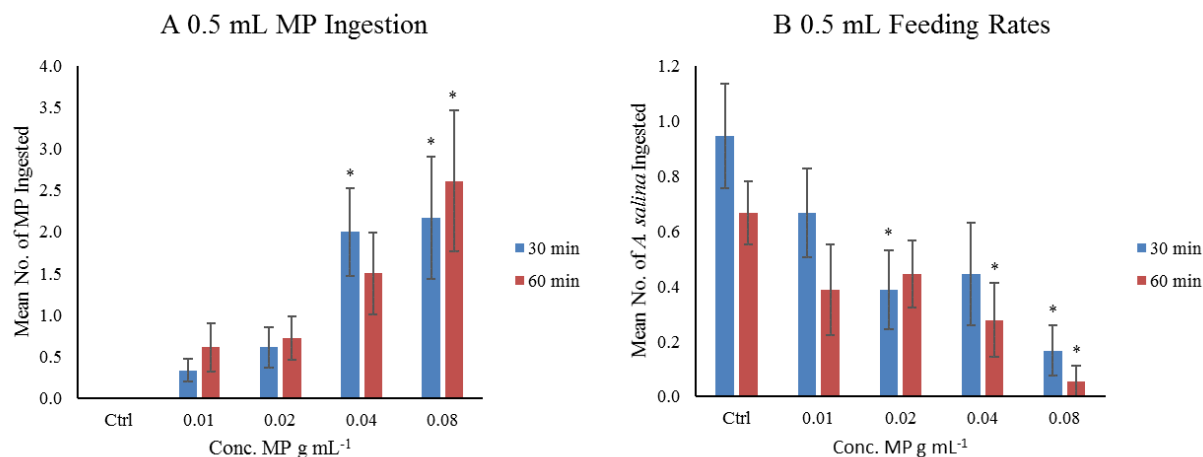


Figure 5.1 (A) mean number of microplastics (MP) ingested by *H. attenuata* in the 0.5 mL exposure at 30 & 60 min time points (error bars = standard error of the mean, * = significance < 0.05) (B) mean number of *A. salina* ingested by *H. attenuata* in the 0.5 mL exposures at 30 & 60 min time points

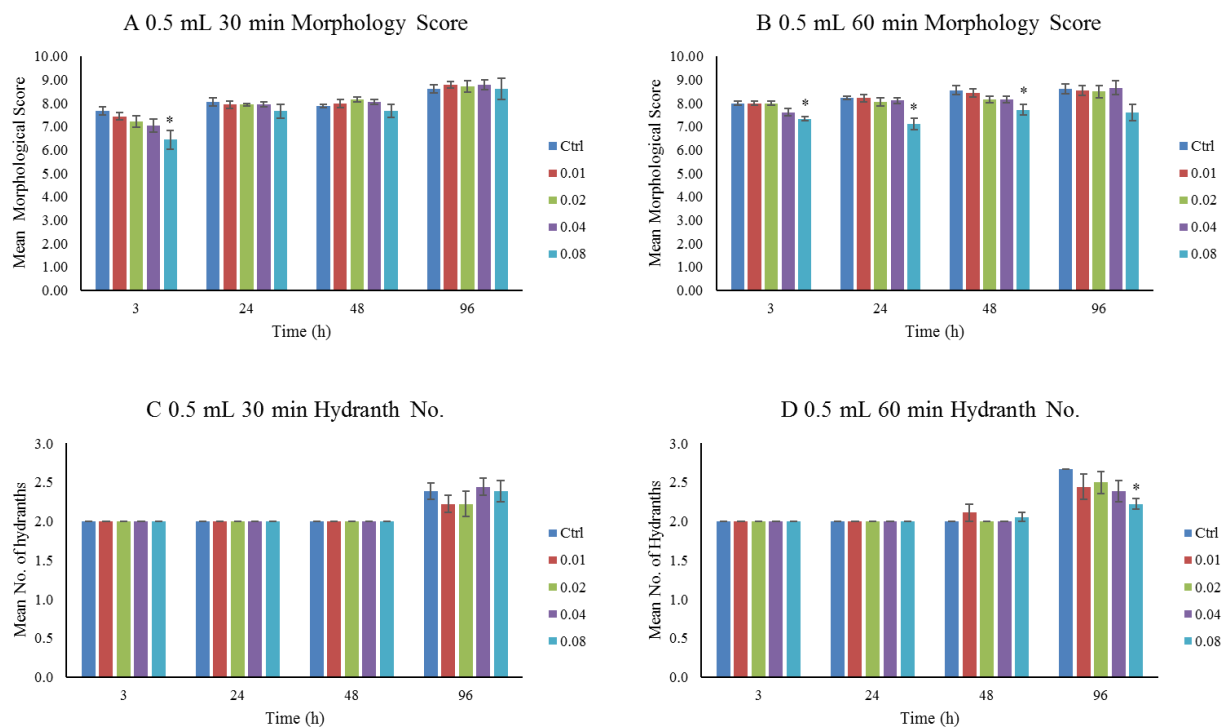


Figure 5.2 Series of bar charts showing (A) mean *H. attenuata* morphology scores for the 0.5 mL 30 min exposures at 3, 24, 48 & 96 hour time points (error bars = standard error of the mean, * = significance < 0.05) (B) mean *H. attenuata* morphology scores for the 0.5 mL 60 min exposures at 3, 24, 48 & 96 hour time points (C) mean *Hydra* hydranth numbers for the 0.5 mL 30 min exposures at 3, 24, 48 & 96 hour time points (D) mean *H. attenuata* hydranth numbers for the 0.5 mL 60 min exposures at 3, 24, 48 & 96 hour time points

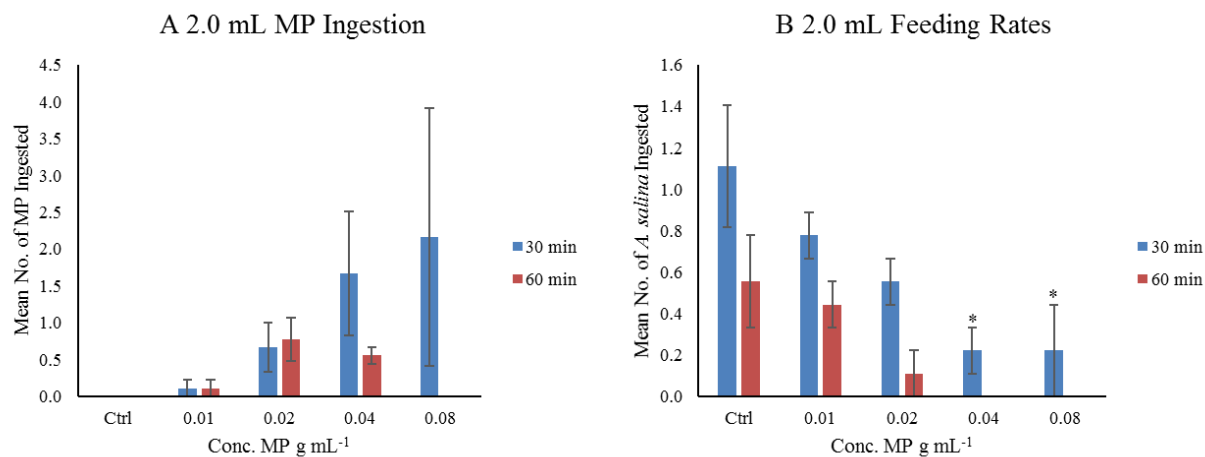


Figure 5.3 (A) mean number of microplastics ingested by *H. attenuata* in the 2.0 mL exposure at 30 & 60 min time points (error bars = standard error of the mean, * = significance < 0.05) (B) mean number of *A. salina* ingested by *H. attenuata* in the 2.0 mL exposures at 30 & 60 min time points

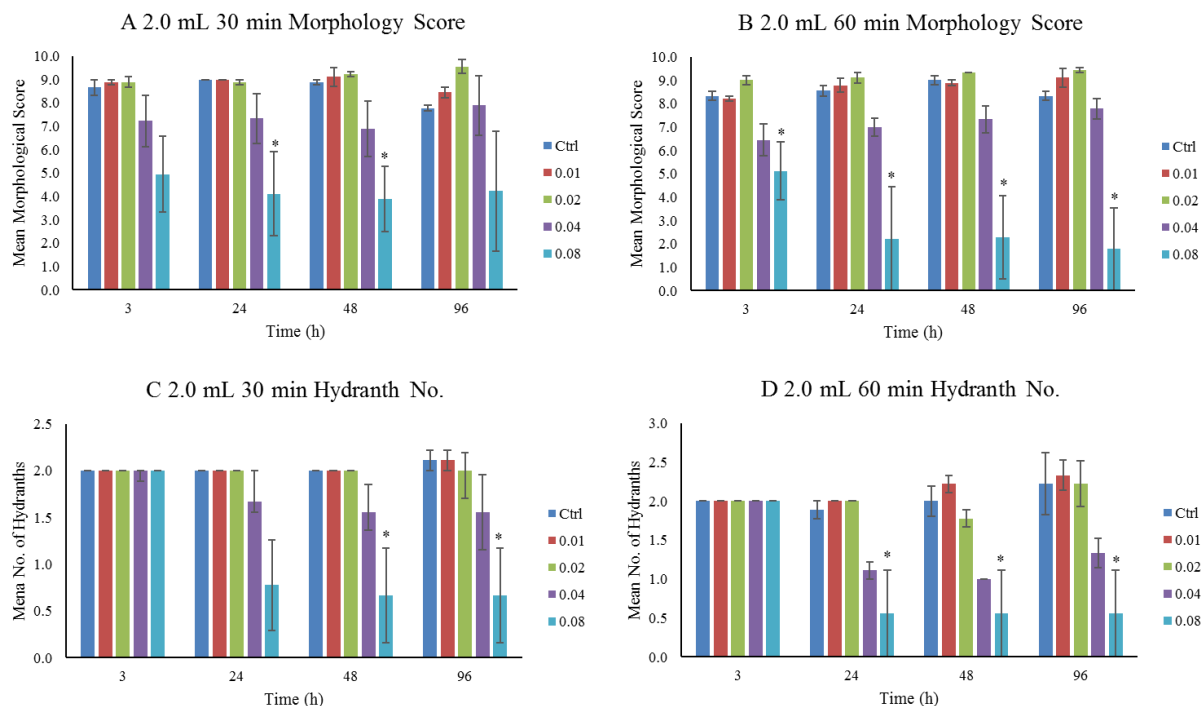


Figure 5.4 Series of bar charts showing (A) mean *H. attenuata* morphology scores for the 2.0 mL 30 min exposures at 3, 24, 48 & 96 hour time points (error bars = standard error of the mean, * = significance < 0.05) (B) mean *H. attenuata* morphology scores for the 2.0 mL 60 min exposures at 3, 24, 48 & 96 hour time points (C) mean *H. attenuata* hydranth numbers for the 2.0 mL 30 min exposures at 3, 24, 48 & 96 hour time points (D) mean *H. attenuata* hydranth numbers for the 2.0 mL 60 min exposures at 3, 24, 48 & 96 hour time points

Table 5.1 The measured environmental concentration (MEC), the predicted no effect concentration (PNEC, extrapolated by dividing the Hydra bioassay IC₅₀ by an assessment factor of 1000) and MEC/PNEC values (used for assessment in Tier two toxicity assessment) for microplastics. MEC values were reported by: a = Lusher et al., (2014) & b = Noren, (2007).

Time	No. of Particles per mL ⁻¹		
	MEC	PNEC	MEC/PNEC
30	0.00000246 a	2.888	0.00000085
	0.102 b		0.04
60	0.00000246 a	2.8	0.00000088
	0.102 b		0.04



Figure 5.5 Photograph of *H. attenuata* with ingested microplastic that can be seen as the blue and transparent particles in the gastric cavity (magnification x25). Due to the buoyancy of the ingested microplastics the foot of this *H. attenuata* was detached from the substrate and the animal was floating.

Chapter 6

Discussion

6.1 Summary of Results

The purpose of this thesis was to investigate various aspects of microplastic pollution from the sources, destination and effects. Chapter 2 presents the results of the first systematic analysis of microplastic in the WwTW process. In Chapter 2 the extraction efficiency of a large secondary WwTW in the removal of microplastic from wastewater was determined and where in the treatment process microplastic was being removed allowing for the identification of the most important steps in microplastic removal and to estimate the amount of microplastic released. In Chapter 3 aquaculture as a potential source of microplastic in the environment was investigated by examining fish cultured for human consumption, wild shellfish and sediment located nearby aquaculture sites for microplastics. The results of Chapter 3 are the first to report microplastic ingestion by cultured fish destined for human consumption. The destination of macroplastic & microplastic in the environment was investigated in Chapter 4 by examining the gastrointestinal tracts of a variety of fish species sampled from Scottish marine waters. The results of Chapter 4 are the first to show that fish in Scottish marine waters are ingesting microplastic with 39.1% of coastal species ingesting microplastic with an additional 9.3% ingesting macroplastic while only 2.4% of offshore species had ingested plastic of any size. In Chapter 5 the effects of microplastic on the feeding, reproduction and morphology of a freshwater organism was investigated by developing a microplastic bioassay. This work described how exposure to microplastic can significantly reduce feeding in *H. attenuata* and that feeding is significant negatively correlated with microplastic ingestion.

6.2 Sources of Microplastic

As part of this thesis two sources of microplastic in the environment were investigated, WwTW and aquaculture. WwTW were known to be releasing microplastic in the environment (Browne et al., 2011, Magnusson & Norén, 2014, Carr et al., 2016) however the extraction efficiency and composition of the microplastic was not fully understood. In Chapter 2 the extraction efficiency of a large secondary WwTW in the removal of microplastic debris was

determined and the amount of microplastic released estimated. Comprehensive identification of the material found in the WwTW liquid fraction as well as the solid fraction of the waste treatment process was also carried out. The identification of microplastic sources is vital in preventing or reducing its release into the environment as once it enters the environment it is very difficult to remove. In order to determine the ability of a WwTW to extract microplastic from the wastewater various points of the WwTW process were sampled. By determining the amount of microplastic present at various points of the WwTW process it was possible to identify where microplastic was being removed and what the most important processes of microplastic removal are.

The influent contained on average $15.70 (\pm 5.23)$ MP. L⁻¹. This was reduced to $0.25 (\pm 0.04)$ MP. L⁻¹ in the final effluent, a decrease of 98.41%. It was found that preliminary and primary treatment effectively removed 78.34% of the microplastic from the liquid fraction. The secondary treatment stage managed to remove a further 20.1%, this involves the removal of biodegradable organic matter as well as suspended solids during the aeration and clarification treatment. Analysis of the solid fraction (grit, grease and SC) samples showed high amounts of microplastic accumulating in these three stages and was most evident in the grease stage, which showed a significantly higher amount of microplastic present. It was only from the grease samples that the much publicised microbeads from face washes were found (Fendall & Sewell, 2009, Napper et al., 2015). Much of the microplastic identified in the WwTW sampled were brittle flakes and fibres which made up > 85% of the microplastics identified (Murphy et al., 2016). The flakes found while numerous were relatively small and brittle and the ease in which they would break may have resulted in their counts being considerably higher than other types found due to fragmentation during filtering and analysis. While fibres have been proven to be released in the final treated effluent in previous studies they were thought to derive from the washing of synthetic clothing (Browne et al., 2011, Napper & Thompson, 2016). Plastic such as polyamide and polyester are used widely in the textile industry to produce clothing. The release of thousands of fibre after a single wash would indicate that this material is a major source of microplastic in the environment (Browne et al., 2011). Microbeads from personal care products do not appear to be capable of passing through WwTW process as previously suggested (Fendall & Sewell, 2009). Due to the majority of microbeads consisting of polyethylene they likely float allowing them to be skimmed off the surface in the grease removal stage. Also, the size of microplastic released in the final effluent mainly consisted of relatively small flakes and fibres so larger microbeads appear unlikely

to be released. There has also been a considerably push to remove microbeads from personal care products and legislation has been create to ban their use (Doughty & Eriksen, 2013, Rochman et al., 2015, Girard et al., 2016). Microplastic fibres released by washing synthetic clothing may be of greater concern than microbeads. However, despite the large reduction of microplastics in the wastewater It was calculated that this WwTW is releasing 65 million microplastics into the receiving water every day which despite the efficient removal rate of the WwTW will contribute significant amounts of microplastic to the environment.

Fibres were also identified in the aquaculture study in Chapter 3 where all microplastic identified were fibres. While aquaculture has been suggested as a potential source of microplastic previously (Cole et al., 2011) little research has been undertaken to determine this, although polystyrene floats used in aquaculture in South Korea were thought to fragment and pollute shoreline sediment (Heo et al., 2013) and aquaculture was suggested as a potential source of polypropylene fibres identified in marine surface waters in Jinhae Bay, South Korea their exact source could not be determined (Song et al., 2015). Aquaculture was investigated as a potential source of microplastics into the environment by analysing cultured fish, wild shellfish located nearby aquaculture facilities as well as sediment. The gastrointestinal tracts of the finfish and the shellfish were examined following procedures used previously (Lusher et al., 2013, Courtene-Jones et al., 2017). Of the two fish species investigated in Chapter 3 for microplastic only one, *H. hippoglossus* was found to have ingested microplastic. Of the *H. hippoglossus* analysed, 60% were found to contain microplastic which is higher than not only the 47.9% of wild *M. edulis* examined but also than the wild fish sampled from Scottish coastal waters in Chapter 4. Although, it's important to state that the number of cultured fish was considerably lower than the wild fish analysed, where only 10 *H. hippoglossus* and 6 *O. mykiss* were analysed. Despite the small sample size the ingestion of microplastic by cultured fish does warrant further research to better understand the threat faced by cultured fish species particularly as the aquaculture is set to grow considerably worldwide over the coming decades (FAO, 2014). Microplastic ingestion has previously been investigated in cultured shellfish with amounts ingested varying study to study (De Witte et al., 2014, Mathalon & Hill 2014, Van Cauwenberghe & Janssen, 2014, Li et al., 2016) and aquaculture has been suggested as a potential source previously (Heo et al., 2013, Song et al., 2015), however there appears to be no research carried out to prove this definitively. While ingestion of microplastic was higher in *H. hippoglossus* than *M. edulis* the microplastic themselves were likely

originating from a similar source as *H. hippoglossus* microplastic consisted of blue (85.7%) polyamide (71.4%) fibres (100%) while the microplastic identified in *M. edulis* were similar blue (94.3%) polyamide (88.6%) fibres (100%). No polystyrene was found in any of the samples despite being reported in sediment thought to originate from nearby aquaculture activity (Heo et al., 2013). The use of polystyrene was not observed in the study area described in Chapter 3, so it is not surprising that none was found and despite the use of polypropylene by the aquaculture sites none was identified in samples despite being suggested as a potential source in a previous study (Song et al., 2015).

Therefore, an attempt was made to determine if aquaculture was a source of microplastic in the environment by comparing rope and netting used in the aquaculture industry to that which was found in the samples examined. By analysing various rope and net samples used by the aquaculture company that provided the fish for this study an attempt was made to compare the microplastics with these rope and net samples to determine if they were the source. Although the material of the rope and netting consisted of similar polymers they did not fully match the physical characteristics of the most abundant microplastic found. Blue polyamide fibres were by far the most common microplastic found in both the cultured fish and wild mussels, only one net sample provided was polyamide however this net was black. Only one rope sample was blue and this consisted of polypropylene. There are other active aquaculture sites that may be using blue polyamide netting or ropes which could be the source of this microplastic, however samples could not be acquired for identification to determine if this was the case. This study is the first to show that cultured finfish are ingesting microplastic and that cultured fish may be affected by the ingestion microplastic at similar or higher rates as wild fish. With 60% of *H. hippoglossus* found to have ingested microplastic which is about 10% higher than what was found in flatfish sampled from the coastal waters of Scotland. However, in order to determine the exact source or sources of microplastic found in Chapter 3 a greater sampling effort of the surrounding area would be needed. This would require surface waters samples to be taken as well as samples of rope and netting of other aquaculture sites as well as samples of effluent from the WwTW identified to be taken in order to determine the sources of the microplastics found.

6.3 Destination of Microplastic

The destination of microplastic in the environment has been widely reported to include aquatic biota (Bellas et al., 2016) as well as shoreline sediment (Browne et al., 2011, Imhof et al., 2013) and deep sea sediment (Van Cauwenberghe et al., 2013a, Woodall et al., 2014) as well as surface waters both freshwater and marine (Law et al., 2010, Eriksen et al., 2013a). In Chapter 4 the destination of microplastic was investigated in the environment by analysing the gastrointestinal tracts of a variety of demersal and pelagic fish from around Scottish marine waters. This is the first study to show the uptake of microplastics by fish in Scottish waters, with four demersal and one pelagic species found to have ingested plastic of some size. The results show that a range of fish species located in several locations in Scottish waters are ingesting macroplastic & microplastic. Ingestion rates of microplastic are as high as 39.1% in some species, rising to 48.4% when all plastic ingested is included. Which is similar to the amounts found in the wild *M. edulis* analysed in Chapter 3 of 47.92% but lower than that found in the cultured fish. There was a clear difference in the ingestion rate between the 2013 and 2014 sampling periods. From the 2013 sampling period, 48.4% of the gastrointestinal tracts of the demersal flatfish contained plastic of some size, with 39.1% having ingested microplastic only and is similar to what has been reported in previous studies (Foekema et al., 2013, Lusher et al., 2013).

The average number of plastic items found per fish was 1.9 (\pm 0.9) with polyamide (65.5%), polyethylene terephthalate (14.7%) and acrylic (14.7%) being the three most commonly found plastics. Polyamide was also the most commonly found polymer in the aquaculture study as well as in the final effluent of the WwTW study (Chapter 2 & 3). Of the 84 pelagic and demersal fish caught in 2014, only 2 (2.4%) individuals from different species had ingested plastic identified as a clear polystyrene fibre and a black polyamide fibre. The higher ingestion rate in 2013 samples is likely due to the samples being taken in shallower coastal waters and consisting entirely of bottom feeding flatfish. While 2014 samples were sampled further offshore in much deeper waters and consisted of a great variety of pelagic and demersal fish with far fewer numbers per species sampled than 2013. It is also interesting to note that of the two aquaculture fish species examined in Chapter 3, only the demersal flatfish was observed to have ingested microplastic while none was found in the pelagic species. It may be that these demersal bottom feeders are more prone to the uptake of microplastic due to their feeding behaviours and preferred habitat. Microplastic may

be accumulating in the sediment where they reside making it far more likely to ingest this contaminant. While no microplastic was observed in the sediment samples collected around the aquaculture site, deep sea sediment has been observed to contain microplastic at depths of up to 4844 m (Van Cauwenberghe et al., 2013a, Woodall et al., 2014). Just four sediment samples were collected in Chapter 3 and the methods of extracting potential microplastic were not ideal. Due to the silty consistency of the sediment conducting density separation (Quinn et al., 2017) was not preformed due to the sediment floating.

The ingestion rates found in Chapter 3 & 4 are all considerably lower than previous studies carried out on invertebrates in Scottish coastal waters (Murray & Cowie, 2011, Welden & Cowie, 2016a, Courtene-Jones et al., 2017), where 83% of *Nephrops norvegicus* were found to contain plastic which is 34% higher than what was found in coastal fish samples in Chapter 4, however later sampling of the same species reported ingestion rates of 67% from three sites sampled around Scotland (Welden & Cowie, 2016a) although ingestion rates varied from 28.7 to 84.1% (Firth of Clyde). While 97% of wild *M. edulis* were found to contain microplastic at nearly twice the rate as the wild *M. edulis* in Chapter 3. The difference in the ingestion of microplastic in the two *M. edulis* species is easier to explain as the same methodology to extract and identify the microplastic was employed so differences in ingestion are likely due to the different areas sampled (Courtene-Jones et al., 2017). The *M. edulis* analysed in this thesis were sampled from an area with high amounts of aquaculture activity which are normally found in unpolluted waters to ensure a quality product is produced. While Courtene-Jones et al., (2017) sampled *M. edulis* from areas nearby a busy maritime town with potential sources from not only aquaculture but also a fishing industry, a ferry terminal and WwTW's all in close proximity to the areas sampled. While the lower ingestion of microplastic by fish compared to *N. norvegicus* is likely due to the different feeding behaviours of the species examined as well as differences in retention time of the microplastic once ingested due to the gut morphology of *N. norvegicus* (Welden & Cowie, 2016a).

6.4 Effects of Microplastic

Both cultured and wild fish are ingesting microplastic in Scottish coastal waters at rates similar to previous studies (Lusher et al., 2013, Van Cauwenberghe & Janssen, 2014). Microplastic ingestion is widespread in species across the world (Besseling et al., 2015, Devriese et al., 2015,

Phillips, & Bonner, 2015, Courtene-Jones et al., 2017). However, the effects of ingestion using environmentally relevant concentrations of microplastic is not well understood (Rochman, 2016) or the potential for microplastic to act as a vector of harmful contaminants (Besseling et al., 2013, Rochman et al., 2013), this is of particular concern due to this widespread ingestion of microplastic.

In Chapter 5 the effects of microplastic were investigated by exposing a freshwater cnidarian to microplastic extracted from a personal care product using a novel bioassay. The results of this study show that *H. attenuata* are capable of ingesting microplastics, with several individuals completely filling their gastric cavities. Significant reductions in feeding rates were observed after 30 min in 0.02 & 0.08 g mL⁻¹ and after 60 min in 0.04 & 0.08 g mL⁻¹ exposures. Exposure to the microplastics caused significant changes to the morphology of *H. attenuata*, however these changes were non-lethal. This study demonstrates that freshwater *H. attenuata* is capable of ingesting microplastics and that microplastic can significantly impact the feeding of freshwater organisms. In this study, *H. attenuata* were observed to have significantly reduced feeding in both the 0.5 mL 30 & 60 min exposures, with feeding rates significantly negatively correlated with microplastic ingestion. Exposure to microplastic has the potential to reduce the health of *H. attenuata* by impacting on its ability to feed and limiting the amount of prey consumed. This interaction could have a profound impact in the environment, not only on wild populations of *H. attenuata* but also on their prey species (Kooijman, & Metz, 1984). Although an impact on the *H. attenuata* morphology in the 0.5 mL microcentrifuge tubes was observed in the present study, these changes were non-lethal and the *H. attenuata* would be able to recover.

During the preliminary exposures, *H. attenuata* were observed to ingest microfibrils which present an interesting opportunity to carry out an exposure using microplastic fibrils. Microplastic fibrils were by far the most abundant type of microplastic observed in Chapter 3 and 4 and made up a substantial portion of the microplastic identified in Chapter 2. Differences in microplastic morphology may potentially result in different rates of ingestion. This was observed in the initial development of this bioassay, *H. attenuata* were exposed to polyethylene microspheres which resulted in no ingestion being observed. While exposure to irregularly shaped polyethylene flakes result in high ingestion rates. The use of microplastic fibrils in exposures studies would make them much more environmentally relevant and provide a much better understanding of the risk faced by

wild organisms ingesting microplastic. Environmentally relevant concentration's of microplastic have been shown to result in negative effects to fish larvae (Lönnstedt & Eklöv, 2016).

6.5 Limitations of this Thesis

6.5.1 WwTW

There were a number of limitations to the research that was carried out in this thesis and are described below. Sampling in wastewater treatment systems presents a number of challenges as reviewed by Ort et al., (2010). This review examined the study of pharmaceuticals and personal care products but should also be applicable to sampling for microplastics. The review highlights practical limitations in sampling such as environmental and the daily variability of flow rates as well as variability in pollutant concentration. In future studies the time of day, year and weather patterns should all be considered when sampling. Due to the great variation of flow rates it may be more appropriate to take frequent samples throughout the day rather than taking a snapshot as was done in Chapter 2. This study did not take into account storm water runoff, where untreated effluent is released directly into the river when the volume of incoming water exceeds the treatable volume. Based on flow rate data taken from the WwTW, when averaged out over the year 39,000 m³ of effluent with limited treatment (settlement in storm tanks) is released every day or potentially an additionally 620 million microplastics/day using the figure of 15.70 MP. L⁻¹ taken from S1. However, this normally occurs in large volumes across short periods of time during spells of bad weather, for example on one particular day over 700,000m³ was recorded to have been released as storm water. This untreated wastewater may potentially heavily increase the amount of microplastic entering the receiving waters.

6.5.2 Aquaculture

The major limitation of this study was the inability to determine if the microplastic identified from the mussel and fish samples were originating from the aquaculture activity. Although ropes sample were analysed from the aquaculture site that provided the fish samples for this study, rope and netting from other sites not involved in this study were unable to be acquired. The small sample size of the fish makes it difficult to determine the extent to which they are ingesting microplastic. While 60% of *H. hippoglossus* were found to be ingesting microplastic

only ten individuals were examined moreover, none of the 6 rainbow trout samples were found to contain microplastic. This is potentially due to their different feeding behaviours and life span but is difficult to determine. A greater sampling effort is needed to determine the extent to which cultured fish are ingesting microplastic. Determine the microplastic concentration in the surface waters surround the sampling sites would have benefited this study as it would have allowed for comparisons between environmental concentrations and the concentrations of microplastic ingested by the mussels and fish.

6.5.3 Ingestion in Wild Fish

Although a variety of species from different locations were sampled around Scotland, the majority of the sample consisted of demersal flatfish from shallow coastal waters. While, deeper water pelagic were sampled, the number of individuals collected per species was considerably fewer. A larger sample size would provide a better understanding of the ingestion rates of these pelagic species. The differences in ingestion may also be due to the different time periods the samples taken from, there was a year between each sampling events. Sampling within a similar time frame or consistent sampling of fish for microplastic ingestion may give a better insight into the threat wild populations face.

6.5.4 *H. attenuata* Exposure

The concentrations used in the *H. attenuata* exposure were not environmentally relevant. The use of high concentrations of microplastic in exposures often does not reflect the reality of environmental concentrations (Cole, 2016, Rochman, 2016). This is an issue as it makes these exposures somewhat unrealistic, however an attempt was made to put the results of the *H. attenuata* exposures into an environmental context by determining the PNEC values and using MEC values to determine if further assessment is necessary. Only one type of microplastic was used in the exposure, blue and clear irregularly shaped polyethylene flakes. While during the course of the study the *H. attenuata* were observed to be able to ingest microfibrils, this study may have benefited from a greater variety of polymers and shapes used. This would have also helped to further validate the usefulness of this bioassay in determining the toxicity of microplastics. While ingestion of microplastic was investigated, this study could have benefited from using microplastic with sorbed contaminants such as POPs or heavy metals to investigate microplastic as a route of other harmful contaminants.

6.6 Future Work

It is clear that microplastics pollution is a growing problem in the marine and freshwater environment considering its pervasive nature as well as the continued input into the environment. It is clear from the studies carried out in the course of this work that microplastic is ubiquitous in the Scottish environment as well as throughout the world as indicated from researching my literature review. Identification of sources of microplastic in the environment is vital in preventing its release. As once it enters the environment it can become very difficult to remove. Future work should focus on the inputs of microplastic within the storm water runoff of WwTW, to determine the potential contribution they are making to the microplastic load of receiving waters. Greater sampling effort is required to determine if aquaculture is a significant contributor of microplastic in marine waters. Continued identification of wild fish species is needed to determine what species are ingesting microplastic and potentially threatened by its presence. Exposure studies need to attempt to use more environmentally relevant concentration as well as different types of microplastics such as fibres to provide more environmentally relevant data.

3130 **Appendix: Research Dissemination**

3131 **Peer-reviewed publication**

3132 **First Author**

3133 Murphy, F., Ewins, C., Carbonnier, F. and Quinn, B., 2016. Wastewater treatment works (WwTW)
3134 as a source of microplastics in the aquatic environment. *Environmental science &*
3135 *technology*, 50(11), pp.5800-5808.

3136 Murphy, F. & Quinn, B., (Submitted). The effects of microplastic on freshwater *Hydra attenuata*
3137 feeding, morphology & reproduction.

3138 Murphy, F., Russell, M., Ewins, C., Quinn, B., (Submitted). The uptake of macroplastic &
3139 microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland.

3140 **Co-author**

3141 Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S.F. and Narayanaswamy, B.E., 2017.
3142 Optimisation of enzymatic digestion and validation of specimen preservation methods for the
3143 analysis of ingested microplastics. *Analytical Methods*.

3144 Quinn, B., Murphy, F. and Ewins, C., 2017. Validation of density separation for the rapid recovery
3145 of microplastics from sediment. *Analytical Methods*.

3146 **Contributed**

3147 Baztan, J., Jorgensen, B., Pahl, S., Thompson, R.C. and Vanderlinden, J.P. eds., 2016. *MICRO*
3148 *2016: Fate and Impact of Microplastics in Marine Ecosystems: From the Coastline to the Open*
3149 *Sea*. Elsevier. "The Effects of Microplastic on Freshwater *Hydra attenuata* Morphology and
3150 Feeding. F. Murphy, L. Prades, C. Ewins and B. Quinn. MICRO2016 Book of Abstracts (p. 78).

3151

3152 **Awards**

3153 2016 SETAC student travel Grant of £250 to attend SETAC Europe 2016 Nantes

3154 2015 University of the West of Scotland Three Minute Thesis Competition

- 3155 • Voted People's Choice
3156 • Runner up winning £500
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3159 **Conference Attendance**

3160 **2016 Marine Alliance for Science & Technology Scotland (MASTS) Glasgow, Scotland**

3161 Workshop: Scottish Microplastic Research Group (SMRG) meeting

3162 **2016 MICRO2016 Lanzarote, Spain**

3163 Talk: The effects of microplastic on freshwater *Hydra attenuata* morphology & feeding

3164 **2016 Society for Environmental Toxicology & Chemistry (SETAC) Europe, Nantes, France**

3165 Poster Spotlight: The effects of the ingestion of microplastics by the freshwater cnidarian,

3166 *Hydra attenuata*

3167 Workshop: Global Water Research Coalition (GWRC) workshop on microplastics in water

3168 **2015 MASTS Glasgow, Scotland**

3169 Workshop: SMRG meeting

3170 **2015 SETAC Latin America**

3171 Abstract: Waste Water Treatment Plants as a source of Microplastics in the aquatic

3172 Environment (Not Attended)

3173 **2015 SETAC Europe, Barcelona, Spain**

3174 Talk: Waste Water Treatment Plants as a source of Microplastics in the aquatic Environment

3175 **2014 MASTS Edinburgh, Scotland**

3176 Talk: Microplastic ingestion in fish collected from Scotland and WWTP as a source of

3177 microplastics

3178 Workshop: SMRG meeting

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